Radiat Environ Biophys (1999) 38: 31–38

ORIGINAL PAPER

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Quantitative modelling of DNA damage using Monte Carlo track structure method

Received: 20 September 1998 / Accepted in revised form: 15 December 1998

Abstract This paper presents data on modelling of DNA damage induced by electrons, protons and alpha-particles to provide an insight into factors which determine the biological effectiveness of radiations of high and low linear energy transfer (LET). These data include the yield of single- and double-strand breaks (ssb, dsb) and base damage in a cellular environment. We obtain a ratio of 4–15 for ssb:dsb for solid and cellular DNA and a preliminary ratio of about 2 for base damage to strand breakage. Data are also given on specific characteristics of damage at the DNA level in the form of clustered damage of varying complexity, that challenge the repair processes and if not processed adequately could lead to the observed biological effects. It is shown that nearly 30% of dsb are of complex form for low-LET radiation, solely by virtue of additional breaks, rising to about 70% for high-LET radiation. Inclusion of base damage increases the complex proportion to about 60% and 90% for low- and high-LET radiation, respectively. The data show a twofold increase in frequencies of complex dsb from low-LET radiation when base damage is taken into account. It is shown that most ssb induced by high-LET radiation have associated base damages, and also a substantial proportion is induced by low-energy electrons.

Introduction

'Microdosimetry' and 'track structure' have been applied to scrutinise and understand aspects of radiation damage in biological molecules from a theoretical approach based upon fundamental physical and chemical principles and provide hypotheses which are testable experimentally. In particular, 'track structure' provides a basis for the understanding of the underlying mechanism(s) that shape dose-effect relationships. There is a wealth of information and data accumulated on radiation biology that need to be placed in the framework of a general, descriptive theory. For example, there are considerable data on ionising radiation tracks; on early effects of radiation on molecular targets such as DNA; on manifestations of DNA damage, after its processing by the cell machinery, in the form of chromosome aberrations, mutational events and genetic instability; on early clonal expansion of the cell to neoplasia; and on the final expression of malignancy.

Over the past decade application of biophysical models to cellular DNA damage has advanced significantly. This has come about through the availability of computer codes describing molecular interactions [1, 2] along the tracks of ionising particles, availability of fast computers and models of DNA in simple and more sophisticated forms [3–5]. These descriptions have progressed from considerations of direct effects of radiation alone [6, 7] to include the contributions of radical species (indirect effect) [3, 8, 9] in the environment of the cell nucleus, causing DNA damage in the form of single-strand breaks (ssb), double-strand breaks (dsb), base damage and complex combinations within the cluster of damage.

Although the majority of ssb are simple if base damage is not considered, a recent important finding is that a high proportion of dsb induced by low-energy electrons are complex [2, 3]. Inclusion of base damage should lead to additional damage complexity, which is the presence of two or more lesions within a few base pairs on DNA. It was hypothesised from biophysical modelling that as the complexity of DNA damage increases, the damage becomes less repairable and hence 'severe', and more likely to lead to biological consequences.

The mechanism(s) involved in radiation oncogenesis are complex, with many steps spanning a long period of time [10]. It is widely believed that the initiating events are due to molecular damage in DNA, primarily in the form of dsb. Over the past two decades experimental work on

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the resulting DNA changes has progressed significantly with the availability of molecular techniques such as PCR (polymerase chain reaction). Theoretical work has also advanced concurrently to address issues related to the mechanism(s) and quantification of initial damage to DNA by ionising radiations of different quality. To this end, track structure has become an important tool in the modelling and calculation of the early effects of radiation in DNA damage induction and provides the experimentalist with the challenge of identifying complex lesions and their influence on damage processing.

This paper presents data on modelling of DNA damage induced by electrons and ions to provide an insight into factors which determine the biological effectiveness of radiations of high and low linear energy transfer (LET). Data are presented on the yields of strand breaks and base damage both from these models and as determined experimentally. From comparison of these approaches, simulations may be fine-tuned to enable meaningful extrapolation to low dose and dose rates which are not readily accessible experimentally. Data are also presented on specific characteristics of molecular damage at the DNA level by ionising radiation, in the form of clustered damage of varying complexity, that challenge the repair processes and, if not processed adequately, could lead to the observed biological effects.

Models of DNA

Models of DNA of varying degrees of sophistication have been used by different workers in the calculation of radiation-induced DNA damage. These can be classified into three groups. The simplest model is a linear segment of DNA in the form of a cylinder. These segments can be generated along random chords cutting a convex body (spherical or cylindrical) using the method of μ-randomness. This model has mainly been used to obtain frequencies of energy depositions in macromolecular structures without *a priori* assumptions of the role of the atomic structure in determining biological responses. A more realistic model used in our studies and others is the volume model [11]. In this model, DNA is in its B-form with a diameter of 2.3 nm and divided into 0.34 nm slices. In turn, each slice is divided into three volumes comprising the central core (representing the volumes of the complementary paired nucleobases) and the two arches (representing the volumes occupied by the deoxyribosophosphate backbone of each strand). This model does not take into account the detailed atomic structure of DNA.

Sophisticated atomic models of DNA have been available for a number of years [12–14]. These structures can now readily be generated using commercially available programmes such as Newhelix [15] and Curves [16]. Further refinements of the latter models include the distribution of solvent molecules around the sugar-phosphate backbone and the nucleobases [4, 17].[1]

[1] Atomic coordinates of a decamer B-DNA including the water of hydration can be obtained from H.Nikjoo@har.mrc.ac.uk

Modelling of higher-ordered structures of DNA, nucleosomes, chromatin and other forms such as triplexes have been made by several authors [18–20]. The majority of these modellings are hypothetical, and these higher-order DNA structures have yet to be confirmed experimentally.

Method of calculation

The starting point of these and similar calculations [21] is simulating the track of the ionising particle, molecular interaction by interaction in DNA and water at 10^{-15} s and establishing the initial distribution of water radicals at 10^{-12} s. Currently, there are a number of computer codes available for the simulation of particle tracks [2]. These distributions are then used to obtain information on the nature of DNA damage induced by the ionising radiation. The usual method is to place the track in a virtual volume big enough to contain the entire track. To sample the track, the DNA is randomly placed in the volume containing the entire track, and a search is made using a 'brute force' technique to locate sites of interaction in the DNA. This method preserves the principle of electronic equilibrium to allow absolute microdosimetric calculations. In principle, we either look for a direct hit on the DNA, in which the co-ordinates, size of the energy deposition and type of interaction are recorded, or the reaction of radical species, diffusing in the environment around the DNA. The mean diffusion distance of radicals in the environment of the DNA is primarily governed by the scavenging capacity of the medium based on the rate constants for the reactions with DNA and with many other reactive molecular species. If there is a reaction between the target DNA and the radicals generated, the position, time and type of reaction are recorded. After scoring the energy deposition events and reactions of radicals, one then searches for possible damage in each DNA segment according to a set of assumptions based on experimental and theoretical information on the mechanisms and pathways to DNA damage.

A major obstacle in scoring damage in DNA is the large cpu time needed for the Monte Carlo track structure calculations. For scoring the long tracks of high energy electrons, alternative methods need to be employed for sampling of the tracks [22, 23].

Calculation of DNA strand breakage

Considerable progress has been achieved recently in the measurement of strand breaks and their distributions in cells (for reviews see [24, 25]). However, the resolution of the experimental techniques is still insufficient for detailed analysis of the hit region of DNA. In calculations of the yields of strand breaks, difficulties with the computational methods remain with the modelling of large macromolecules, tracing the target to find sites of interactions [23] and the primary data on the pathways to strand breakage. Table 1 provides a summary of selected experimental data

Table 1 Summary of selected experimental data for the yield of single- and double-strand breaks (ssb, dsb) for low- and high-linear energy transfer (LET) radiation (*RBE* relative biological effectiveness, *USXR* ultrasoft x-rays)

Reference	Radiation	LET (keV/µm)	ssb/Gy/Da $\cdot 10^{-10}$	dsb/Gy/Da $\cdot 10^{-11}$	eV/ssb[a]	eV/dsb[a]	RBE_{dsb}	ssb/dsb	Medium	Comments
Neary et al. 1972 [52]	Proton	22	2.33	1.07	44.4	970	1.02	22	Solid DNA	
	α-particle	50	2.18	1.14	47.5	906	1.09	19	Solid DNA	
	α-particle	90	2.72	1.05	38.1	985	1	26	Solid DNA	
Frankenberg et al. 1981[53]	α-particle	113	–	1.5	–	66	2.6	–	Yeast	
	Electron	–	–	0.6	–	170	–	–	Yeast	
Frankenberg et al. 1986 [54]	Al_K USXR	–	–	0.9	–	106	2.2	–	Yeast	
	C_K USXR	–	–	1.6	–	62	3.8	–	Yeast	
Lett et al. 1987 [55]	Hard x-ray	–	–	–	60	1000	–	10	V79	
Siddiqi & Bothe 1987 [56]	γ-ray	–	–	–	–	–	–	19	Dilute sol	
Baverstock & Will 1989 [57]	^{125}I	–	–	–	–	–	–	10	Solid DNA	
O'Neill et al. 1997 [58]	γ-ray	–	4	1.67	–	–	–	40	Plasmid	
	γ-ray	–	2.9	0.88	–	74	–	32	V79	
	Al_K USXR	–	<3.6	1.6	–	–	1	22	Plasmid	0.1 M Tris
Botchway et al. 1996 [59]	Al_K USXR	–	2.6	2.2	–	233	2.5	12	V79	
	Cu_L USXR	–	–	2.6			3.0		V79	
Jenner et al. 1992 [60]	p & α	21.5		–	–	445	1.08	–	V79	
	α-particle	120	0.7	0.73			0.8	10	V79	
Folkard et al. 1993 [39]	Electron	–	2.51	2.6	25	25–50		10	Solid DNA	2 keV e^-
Heida et al. 1986 [61]	Synch. radiation	–			7	–			Solid DNA	
Ito 1987 [62]	Synch. radiation	–			7.5	–			Solid DNA	
Michael et al. 1995 [63]	Synch. radiation				8	8			Solid DNA	

[a] Quantities in these columns are not all directly comparable, representing in some cases the average energy to the sample and in other cases the local energy at the DNA break

for DNA and cellular DNA. Data are given for the yields of strand breaks, the ratio of the number of ssb to dsb, the relative biological effectiveness for dsb formation relative to ^{60}Co gamma-ray, and some data on the amount of energy required for the induction of a ssb and a dsb assuming a single track effect.

Calculation of the yield of strand breaks using the Monte Carlo techniques requires knowledge of a number of parameters, including (a) absolute frequency of energy deposition in a segment of DNA per unit dose of the radiation under test; (b) the probability of energy deposited in DNA inducing a ssb or dsb; (c) the efficiency of a hydroxyl (OH) or other radical species in inducing a strand break; and (d) the separation between two ssb that constitutes a dsb.

Using the assumptions for the simulations discussed below, a main feature of existing models is that the calculated yields of strand breaks are in reasonable agreement with experimental data. For instance, the experimentally measured yields of ssb and dsb produced by Al_K ultrasoft x-rays (USXR) in V79 cells are similar to the corresponding calculated yields of breaks by 1.5-keV electrons (Tables 1, 4). The yield of strand breaks is a good parameter to check the consistency of the method but does not tell us much about the complexity of DNA damage. To probe details of the complexity of damage in the hit region of a DNA segment, we have used a model of DNA damage which classifies strand breaks according to the lesion distribution and the source of damage [3], namely: (a) the source of damage, (b) the complexity of damage considering only strand breaks and (c) contributions of base damage to the complexity model in (b). The classification for the source of damage (description a) considers the strand breaks arising either from direct energy deposition (subscript D), or originating from the reaction of hydroxyl radicals and other indirect radical species (subscript I), with the sugar-phosphate and the nucleobases. The classification of damage according to complexity (description b) provides classifications for a ssb only on one strand (SSB), two ssb or more on the same strand (SSB^+), and two or more ssb on opposite strands but not constituting a dsb (2 SSB). Similarly, dsb can also be considered as simple (DSB), complex with one double strand and one or more additional strand breaks on one strand only (DSB^+), and the most complex class involving at least two dsb in the region of the hit of the DNA (DSB^{++}). This classification (b) does not distinguish between the sources of the break (D or I). Model (c) provides classification of additional damage according to the number of base damages in each of the classifications of strand breakage by complexity. Base damages were divided into groups of 0, 1, 2, 3, 4, 5 and >5 damaged bases in the DNA segment. The overall estimation of base damage has been presented as the ratio of the number of base damages (BD) to strand breaks assigned as SPD.

In these calculations it is assumed that a strand break arises from either an energy deposition of $E_{ssb} \geq 17.5$ eV by a single track of the particle in the sugar-phosphate or an OH radical which has a probability of 0.13 [26] of causing a ssb on reaction with DNA. This value of 0.13 is notionally composed of the probability of 0.2 of reacting with the sugar-phosphate rather than the nucleobase and the probability of 0.65 of this interaction leading to a break. Calculations have been performed to investigate the sensitivity of the yields to variations in the values of these two parameters [3]. It is assumed also that a dsb is formed if

Table 2 Variation of the relative yield of dsb with OH activation probability for 1.5-keV electrons

P_{OH}	DSB_I (%)	DSB_D (%)	DSB_{hyb} (%)
0.001	–	99	1
0.002	1	95	4
0.04	1	92	7
0.1	8	79	13
0.13	12	73	15
0.2	23	59	17

the breaks on opposite strands are within ≤10 base pairs separation.

We have used the estimated mean diffusion range of radicals of about 4–6 nm in a cell environment [3]. Contributions of hydrogen atoms and hydrated electrons to DNA strand breakage were ignored as these were considered not to cause appreciable strand breaks. Similarly, base to sugar radical transfer from damage induced by OH radicals or from direct energy depositions has not been included except for any contribution inherent in the experimental probability of 0.13 for OH radicals to yield a ssb.

Table 2 shows the variation of the relative yield of dsb with the OH activation probability for 1.5-keV monoenergetic electrons. The last column gives the yield of ssb converted to dsb by OH radicals. For the preferred value of $P_{OH} = 0.13$, the contribution of the direct effects for dsb yield is probably greater than that suggested experimentally. The limiting case of $P_{OH} = 0.001$ mimics the situation of direct effect only, when OH radicals are entirely absent from the system or are entirely scavenged before reacting with DNA.

The first of the required parameters listed above is the absolute frequency distribution of energy depositions per unit dose in the volume of the target such as DNA. The database for the frequencies of energy deposition in a small volume corresponding to the biological targets for electrons, x-photons and ions, and the methods of the calculation can be found in various publications [22, 27–29]. The frequencies of energy deposition can also be generated using an algorithmic method [30].

The second of the parameters in elucidation of DNA damage is the size of energy deposition in DNA to cause a strand break. Analysis of experimental data shows a linear dose relationship for the dsb yield. Assuming a Poisson distribution of breaks, then the mean number of breaks per dose is a constant ($y_{dsb} = n_{dsb}D$). This also implies that dsb are produced predominantly by single track events. The yield of dsb per Gy per dalton can then be used, under certain experimental conditions, to estimate the overall average energy to the system required for the production of a dsb. If n_{dsb} is the number of dsb per Gy per dalton and MW the molecular weight, then the mean energy in eV per break is $(MW)/6.023 \cdot 10^{23} \cdot 1.602 \cdot 10^{-12} \cdot n_{dsb} = 1.0364 \cdot 10^{-10}$ $(MW)/n_{dsb}$. It is on such a basis that the larger values in Table 1 (columns 6 and 7) were calculated from experimental data.

The above relationship is not a satisfactory basis for the calculation of the energetics of DNA damage. To establish a criterion for the local energy required to induce a ssb, numerous values have been used in the literature based mostly on their physical and chemical significance. These include average energy loss, ionisation energy, oscillator strength and average excitation potential ranging from 12 to about 30 eV. To obtain a more realistic value based on experimental data [11, 31–33], experiments were simulated in which the DNA fragment length distributions were measured after decay of incorporated ^{125}I [34, 35]. These calculations provided an experimentally based average value of 17.5 eV in the sugar-phosphate moiety, based on best agreement between empirical fitting of the experimental

Table 3 Examples of energetics of DNA damage used in various calculations

Reference	Method	Energy for induction of 1 SSB	Energy required for induction of 1 DSB	Target size	Comments
Goodhead & Brenner 1983 [46]	Track structure: correlation with expt.	–	>100 eV	Sphere (D = 3 nm)	Average energy deposited in a DNA size target
Goodhead & Nikjoo 1988 [11]	Track structure: assumptions	>30 eV	>100 eV	Cylinder (2 nm×2 nm)	Average energy deposited in a DNA size target
Charlton & Humm 1989 [34]	Track structure: expt. fit with model	>17.5 eV	>35 eV	≤10 bp sep. bet. 2 SSB B-DNA	Simulation of experiment
Holley et al. 1990 [7]	Track structure: Fricke assumptions	29.9 eV 17 eV/OH	60 eV	≤10 bp sep. bet. 2 SSB B-DNA	Oscillator strength of DNA
Terrissol & Pomplun 1992 [34]	Track structure: expt. fit with model	>18 eV	>36 eV	≤10 bp sep. bet. 2 SSB B-DNA	Simulation of experiment
Nikjoo et al. 1996 [33]	Track structure: expt. fit with model	>17.5 eV	>35 eV	≤10 bp sep. bet. 2 SSB B-DNA	Simulation of experiment
Friedland et al. 1998 [20]	Track structure: expt. fit with model	>10.5 eV	>21 eV	≤4 bp sep. bet. 2 SSB B-DNA	Simulation of experiment
Brenner & Ward 1992 [49]	Track structure: correlation with expt.	–	2 ionisations	Spherical cluster (D = 2 nm)	Cluster analysis
Nikjoo et al. 1996 [33]	Track structure: expt. fit with model	2 ionisations	4 ionisations	10 bp sep. bet. 2 SSB B-DNA	Simulation of experiment

Table 4 Relative yield of strand breaks according to the source (direct and indirect)

Energy	SSB_I %	SSB_D %	DSB_I %	DSB_D %	ssb/Gy/Da $\cdot 10^{-10}$	dsb/Gy/Da $\cdot 10^{-11}$	SSB/DSB	BD/SB
Electrons								
0.3 keV	34	66	29	71	2.5	2.3	13	2.0
1.5 keV	39	62	27	73	2.4	1.8	13	2.2
4.5 keV	34	66	23	76	1.9	1.2	15	2.0
Protons								
0.5 MeV	39	61	33	67	2.3	3.2	7	2.2
1.0 MeV	40	60	38	63	2.4	2.6	9	2.3
4.0 MeV	40	60	39	61	2.5	1.8	13	2.4
α-particles								
2.0 MeV	46	54	31	69	1.7	4.0	4	2.0
6.0 MeV	40	60	29	71	2.2	3.7	6	2.1
10.0 MeV	42	59	35	65	2.3	2.9	8	2.2

data with track structure simulations. Table 3 gives a summary of the data used in various track structure studies. In one recent study, a value was used of 10.5 eV within two van der Waals radii of all atoms of the sugar-phosphate moiety, based on fitting experimental yields of dsb from 220 kVp x-rays with associated assumptions that two breaks within ≤4 base pairs separation are required for a dsb and ignoring indirect effects [20]. By contrast, values obtained from a series of experiments using synchrotron radiation seem to indicate a much lower threshold energy of about 8 eV needed to induce a ssb (Table 1) [36–38], but experiments using very low energy electrons indicated a higher threshold of up to about 25 eV (Table 1) [39]. However, a detailed analysis and modelling of the experiments using synchrotron radiation or electrons have yet to be done. Calculated absolute yields for ssb and dsb per Gy per dalton, as well as relative yields, are presented in Table 4 for various radiations, using our preferred parameters.

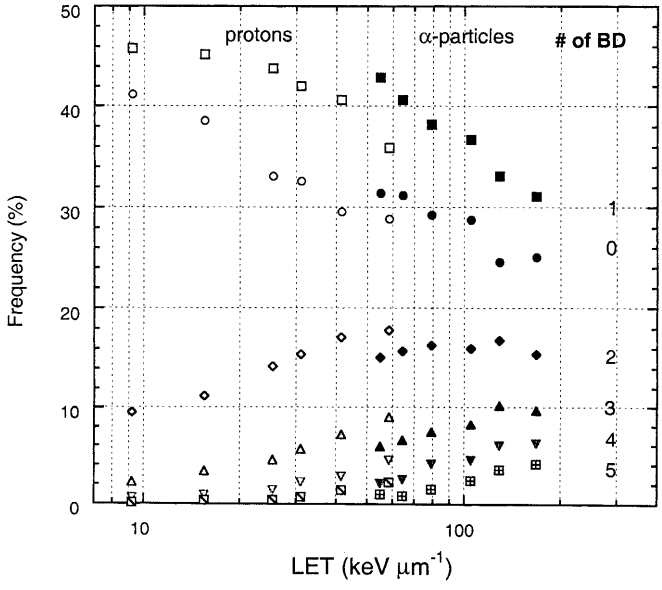

Fig. 1 Frequency of base damage for protons and alpha-particles as a function of LET. Data are presented according to the number of base damages in the hit region of the DNA segment. Numbers listed under *# of BD* refer to the segments containing 0, or 1, 2, … and 5 damages. Data for segments containing more than 5 base damages are not explicitly shown

Calculation of base damage

The calculations of base damage were based on the minimum energy to produce base damage by direct energy deposition and the probability of an OH radical giving a base damage. The classification of numbers of base damages is described in the above section on the yield of strand breaks. As an initial approach for the estimation of base damage, the same criterion as for the induction of direct strand breaks was adopted, *viz* ≥17.5 eV for the energy deposition by direct interaction of the track with the nucleobases, and for indirect effects a probability of 0.80 was assumed for induction of base damage from the reaction of OH radicals with DNA to include all OH reactions with nucleobases. In the calculations, transfer of charge to a preferential base was not considered explicitly on the assumption that transfer occurs only over a few base pairs. Figure 1 shows the frequencies of base damage as a function of LET of the particle. Data are presented according to the number of base damages in the hit region of the DNA. The data show that for low-LET radiation about 60% of the DNA hits contain at least 1 base damage, and the total yield increases with increasing LET, as does the degree of complexity. There are apparently more damaged bases than strand breaks, with an estimated ratio of 2.0–2.4 for a variety of radiations (Table 4). Table 5 shows the increase in proportion of complex ssb and dsb when base damage is taken into account in this way.

To obtain a more realistic picture of complex DNA damage, it is necessary to include base damage, as the above initial approach has shown. It is envisaged that significant refinements to the simulation of base damage will be the focus of future approaches on DNA damage. For instance, for direct energy deposition events in DNA, the ionisation potential of the bases could be used as an energy threshold for ionisation. With this threshold and using an OH radical probability of 80% to give a base modification, the

Table 5 Relative yield of strand breaks according to complexity

Energy	No break (%)	SSB (%)	SSB^+ (%)	2 SSB (%)	DSB (%)	DSB^+ (%)	DSB^{++} (%)	SSB_c (%)	SSB_{cb} (%)	DSB_c (%)	DSB_{cb} (%)	SSB_{cb}/SSB_c	DSB_{cb}/DSB_c
Electrons													
0.3 keV	66.41	26.55	3.29	0.42	2.38	0.85	0.091	12	52	28	68	4.3	2.4
1.5 keV	70.45	24.33	2.41	0.41	1.69	0.63	0.074	10	41	30	60	4.0	2.0
4.5 keV	71.39	24.13	2.13	0.29	1.47	0.55	0.041	9	37	30	62	4.0	2.1
Protons													
0.5 MeV	60.40	26.31	5.38	1.10	3.89	2.29	0.62	20	70	46	86	3.0	1.6
1.0 MeV	66.59	24.82	3.70	0.66	2.67	1.13	0.33	15	59	37	80	4.0	2.2
4.0 MeV	73.58	21.71	2.09	0.45	1.63	0.45	0.08	10	45	26	66	4.5	2.5
α-particles													
2.0 MeV	51.30	23.00	7.00	1.97	4.8	6.20	5.70	28	75	73	96	2.6	1.3
6.0 MeV	58.45	21.51	5.09	1.16	3.45	2.67	1.24	21	70	56	90	3.0	1.4
10.0 MeV	64.30	24.80	4.40	0.98	3.20	1.90	0.40	18	65	45	84	3.5	1.6

yield of nucleobase radicals is obtained. Since not all base radicals are converted to persistent, diamagnetic damage, a further probability factor is required to convert a base radical into a damage. These may be derived from experimental data on radiation-induced base damage. For instance for gamma-radiation, the OH radical-induced yield of base damage has been measured to be 2–3.8 times greater than that for ssb under aerobic conditions [40]. From direct effects [41] and assuming the yield of base release corresponds to that for ssb, it is estimated that the yield of base damage is nearly twice that for ssb. Unfortunately, significant experimental data on the yield of base damage for different radiations are not available. Therefore, a future approach to simplify the simulation of base damage could be to estimate its contribution from direct and indirect effects for the different radiations from the respective yields of ssb and using the experimental base-damage/ssb ratios above.

Complexity of DNA damage

There have long been indications that the biological consequences of ionising radiations are determined by their clustering properties at the level of the DNA duplex [42–44]. From the database on the frequencies of energy deposition in volumes of biological dimensions, it became possible to seek and correlate the size of energy deposition with particular biological effects [45]. An early application of track scoring was made for x-rays of various energies, which indicated that biologically critical properties are in the regions greater than 100 eV of energy deposition in volumes similar to that of DNA [46]. In a similar manner the dominant feature associated with high-LET radiation was found to correspond to a class of clusters of energy depositions greater than about 300 eV in nucleosome size targets [45]. The above approaches could not give information on the molecular nature of the DNA damage, but they indicated the need for more detailed simulations at the molecular level.

Table 5 provides a relative distribution of damage in terms of its complexity. Data are presented for simple and complex strand breaks induced by electrons, protons and alpha-particles of various energies. Complex ssb are defined as those segments of DNA containing a ssb with an associated break(s) on the same or the opposite strand ($SSB_c = SSB^+ + 2\ SSB$). Similarly, complex dsb are defined as $DSB_c = DSB^+ + DSB^{++}$. To account for the contribution of base damage (BD), SSB_{cb} is defined as the sum of all ssb containing at least one base damage in the hit region of the DNA. Similarly, for the dsb, frequencies of complex dsb including base damage is calculated as $DSB_{cb} = DSB_c + DSB_{bd}$ where DSB_{bd} are those DSB with at least one base damage not included in DSB_c. It is seen from Table 5 that inclusion of base damage substantially increases the complex proportions of both ssb and dsb.

Discussion

The criteria for the induction of strand breaks were described assuming a threshold energy deposition for direct interaction of the track and a probability for the reaction of hydroxyl radicals with DNA. The method was extended to estimate the yield of base damage, in the first instance adopting the same criteria as in the direct induction of strand breaks and an appropriate probability factor for the reaction of the hydroxyl radical with the nucleobases causing a base damage. A summary of the energetics of DNA damage is given. These were divided into two groups: those derived from physical and chemical properties of liquid water and DNA, and those derived from a knowledge of experimental data of DNA strand breakage. Use of incorporated Auger emitters in DNA, particularly ^{125}I radionuclide in plasmids, has made it possible to examine more extensively the scission of the DNA strand and its energetics [33, 47, 48]. Biophysical modelling of DNA damage has proposed the use of a quasi-threshold energy for simulation of the induction of a ssb [31]. For most modelling

to date, this value has been set at $E_{ssb} \geq 17.5$ eV based on experimental data with ^{125}I. Other calculations, for modelling of DNA fragment length distributions by external beam irradiation, have used 10.5 eV based on experimental data for x-rays and different associated assumptions [20]. Ionisation of DNA as an alternative measure for the induction of DNA strand breaks has also been used in a number of calculations [49, 50]. However, inherent in the use of such a measure is the assumption that ionisations within the nucleus have a low probability of inducing dsb (about 1 in 1500 [51]). Similarly, if assuming every ionisation has the capability of leading to a dsb then, using the rejection method, the breaks can be sampled to match the experimental yield of 30–40 dsb per Gy of radiation. On the other hand, the low-energy electron and photon experiments [36–39] have posed new questions on the energetics of DNA damage and the possible role of the super excited states of DNA leading to strand breakage. The results of the latter experiments are important as the photon energy is below the ionisation potential of the DNA.

In summary, data presented on the complexity of DNA damage show that:

- The calculated yield of damage is similar in magnitude to the experimental data.
- In the cellular environment, the majority of damage results from direct energy deposition in DNA, but the contributions from hydroxyl radicals are substantial.
- The ratio of ssb/dsb is nearly 4–15 for solid and cellular DNA, similar to the finding from experimental data.
- Ratio of base damage to strand breakage is initially estimated as about 2 for various radiations. Similar values have been deduced from experimental results with low-LET radiation in model systems.
- Analysis of data shows that clustered damage occurs at high frequencies. Nearly 30% of dsb are of complex form for low-LET radiation, rising to about 70% for high-LET, solely by virtue of additional associated breaks.
- Inclusion of base damage increases the complexity, apparently raising the complex proportions for low-LET radiation to about 60% and for the high-LET to about 90%. This shows a twofold increase in the frequencies of complex dsb from low-LET radiation when base damage is taken into account.
- Similarly, base damages are associated with most ssb induced by high-LET radiation, and a large proportion (nearly 45%) induced by low-energy electrons have associated base damage.

Acknowledgements This work was partly supported by the European Community under contract no. F14P-CT95-0011c (Biophysical Models for the Induction of Cancer by Radiation). Thanks to Norma King and Anne Johnson for helping with the preparation of the manuscript.

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