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Measurement of DNA damage by electrons with energies between 25 and 4000 eV

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Abstract. All ionizing radiations deposit energy stochastically along their tracks. The resulting distribution of energies deposited in a small target such as the DNA helix leads to a corresponding spectrum in the severity of damage produced. So far, most information about the probable spectra of DNA lesion complexity has come from Monte Carlo studies which endeavour to model the relationship between the energy deposited in DNA and the damage induced. The aim of this paper is to establish methods of determining this relationship by irradiating pBR322 plasmid DNA using low energy electrons with energies comparable with the minimum energy thought to produce critical damage. The technique of agarose gel electrophoresis has been used to ascertain the fraction of DNA single- and double-strand breaks induced by monoenergetic electrons with energies as low as 25 eV. Our data show that the threshold electron energy for induction of single-strand breaks is <25 eV, and for double-strand breaks between 25 and 50 eV.

1. Introduction

Recent studies of the biophysical mechanisms of radiation damage have emphasized the importance of the microscopic structure of individual radiation tracks on a scale comparable with the DNA helix (Charlton *et al.* 1989, Goodhead 1989, Holley and Chatterjee 1990, Nikjoo *et al.* 1991). Using Monte Carlo track structure techniques, it has been shown that virtually all radiations, even those initially monoenergetic, will deposit a wide range of energies within the critical target (i.e. a small section of the DNA helix), from zero energy, up to a few hundred electron-volts, although densely ionizing radiations will produce a greater fraction of large energy depositions than will sparsely ionizing radiations. One of the interesting findings from the track structure work is that the efficiency of different radiations to produce energy depositions of about $\geqslant 100$ eV within a small segment of DNA correlates reasonably well with their experimentally measured relative biological effectivenesses (RBEs) for cellular inactivation (Goodhead 1989). However, small energy depositions (less than a few tens of eV) occur with an efficiency that is the inverse of the RBEs for cellular inactivation, although they do correlate with the production of DNA single-strand breaks (ssb). It is concluded from these observations that an energy deposition of about 100 eV within the DNA helix represents a 'quasi-threshold' for the production of lethal damage (i.e. a DNA double-strand break (dsb)) and that energy depositions much less than this are unlikely to produce critical damage.

There is, as yet, very little direct experimental evidence to support many of these theoretical findings. The work presented here is an attempt to measure directly the threshold energies for the production of DNA ssbs and dsbs. We have developed an electron source capable of producing electrons with any energy between a few and 4000 eV. The source has been used to irradiate dry pBR322 plasmid DNA in vacuum. Owing to the very short range of electrons, we have also developed techniques for preparing the DNA sample as near as possible to a monolayer. The undamaged plasmid exists in a supercoiled topological form (form I). If a ssb is induced, then the plasmid converts to a relaxed form (form II). If a dsb is produced then the plasmid changes to linear form (form III). Using the technique of agarose gel electrophoresis we have measured the fractions of these forms induced by monoenergetic electrons with selected energies between 25 and 4000 eV.

Photons remain the radiation of choice for experiments of this type as they are the most penetrating. Unfortunately, photons that are sufficiently monoenergetic in the vacuum UV region cannot be generated using low-cost equipment with sufficient intensity. By comparison, low energy electrons are relatively straightforward to produce. However, the inferior penetration offered by this radiation (only a few nanometers below about 100 eV) places stringent demands on the preparation of samples. Experiments using synchroton-generated UV photons

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down to 10 eV are under development and will be presented in the near future. Before this, however, we have completed preliminary experiments to measure the induction of DNA damage using low-energy electrons. Assuming that the effects are due only to single-track events, then the maximum energy deposited in a segment of DNA can be no greater than the initial energy of the radiation. Note, however, that all electrons, irrespective of their energy, deposit very few clusters >200 eV (Nikjoo *et al.* 1991). Note, also, that <100 eV only a few inelastic events per electron are likely. Measurements of energy loss distributions in organic materials (Rauth and Simpson 1964) reveal a skew distribution with the most frequent energy loss per event at 22 eV (however, the mean energy loss per event is 60 eV). Similar findings are obtained by Johnson and Rymer (1967) from measurements of the energy loss distributions of 150 keV electrons in nucleic acid. A 25 eV electron is therefore likely to produce no more than one ionization.

Previous work on the biological effects of low energy ionizing radiations has been confined largely to studies using ultrasoft X-rays, with energies of ⩾278 eV (the carbon-K edge). One important conclusion from both the experimental (Goodhead *et al.* 1979, Folkard *et al.* 1987, Prise *et al.* 1989) and theoretical (Goodhead and Nikjoo 1989) studies is that very localized clustering of energy depositions are efficient at producing lethal damage. Much data exist on the effects of low-energy electrons >500 eV, due largely to the work of Cole *et al.* 1980 and references therein) who exploited the limited range of electrons to investigate differences in spatial sensitivity across the cell nucleus.

Very little previous work exists using ionizing radiations with energies below the carbon-K edge. Setlow (1960) showed that there is a rapid rise in the inactivation efficiency of enzymes by photons >10 eV, which is attributed to the onset of ionization. A similar conclusion was reached by Hutchinson (1954) from studies of the inactivation of proteins by electrons with energies ranging from 5 to 200 eV. Lücke-Huhle and Jung (1973 a, b) used metastably excited gases from 4·3 to 19·8 eV to induce strand-breaks in ϕX174 single-stranded and ϕX174 RF double-stranded DNA. They concluded, however, that the energy transfer mechanisms do not involve ionization and their results are therefore not readily applied to our investigation. Other work (Wirths and Jung 1972, Sontag and Dertinger 1975) using ϕX174 DNA with UV photons in a similar energy range shows that the quantum yield of strand-break production increases by about 10^5 on increasing the photon energy from 5 to 10 eV, beyond which this quantity is practically independent of energy. Also, a correlation is shown between this result and the quantum yield of electron emission from DNA (indicative of ionization).

2. Methods

2.1. *Source construction and dosimetry*

A schematic diagram of the electron source is depicted in Figure 1 and comprizes a 75 × 115 mm diameter brass chamber supported vertically with a removable base for loading and unloading samples. Within the chamber at the upper end is a ceramic plate supporting a tungsten filament extracted from a 1·5 V torch bulb. The filament is energized by a variable power supply, floating with respect to ground potential and tied to the accelerating voltage. The accelerating potential is provided by a second power supply, variable from zero to −4000 V. The energy of the electrons (expressed in electron-volts) striking the sample will be numeri-

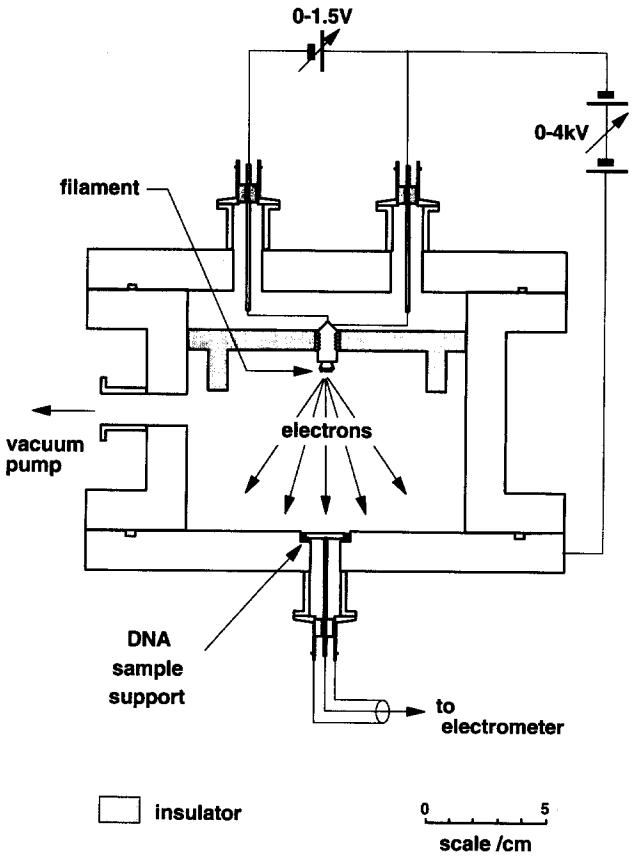

Figure 1. Apparatus for irradiation of thin films of DNA with low-energy electrons.

cally the same as the accelerating potential, but with a 0–1·5 eV energy spread above this due to the potential across the filament. Isolation amplifiers in the filament circuit are used to stabilize and regulate the filament output (and hence dose-rate). They also provide ground-referenced indications of the filament current and voltage.

The prepared sample (see § 2.2) is supported on a 24 mm diameter brass disc within the base, but separated from the rest of the base by a small insulating gap. Both the disc and base are at ground potential. The sample dose is calculated from the measured flux (i.e. the number of the electrons per unit area per s) at the sample position, and the irradiation time. The current due to the electrons irradiating the sample is integrated using a current-to-voltage converter followed by a voltage-to-frequency converter coupled to a 4·5-decade calibrated counter. The counter is arranged to terminate the irradiation when a predetermined charge is reached. Reliable dosimetry using low energy electron beams is complicated by a number of factors; electron flux, rate of energy loss, dE/dx, and hence dose, will vary with depth into the sample. This is true even if the sample layer is only a few nanometers thick. Furthermore, published range and stopping power measurements (correlated in a paper by Iskef *et al.* 1983) show large uncertainties in these quantities < 100 eV. Despite this we have endeavoured to assign a surface dose, in Gy, delivered to all the irradiated samples, so that comparisons of the effects of different energy electrons are based on equivalent energy depositions, rather than exposure. The measured charge, Q, has been converted to dose, D, using the stopping power data for electrons in collodion (which has an electron density similar to nucleic acid), obtained experimentally by Cole (1969), i.e.

$$D = \frac{Q\rho}{eA}\frac{dE}{dx},$$

where e is the electronic charge, A is the charge collection area and ρ is the ratio of the densities of nucleic acid and collodion. All dose calculations are based on the incident electron energy.

2.2. *Sample preparation*

The very short range of low energy electrons dictates that the plasmid DNA is irradiated in a dry condition, prepared as a monolayer, or as near to a monolayer as can be achieved. Our ability to meet this requirement is discussed in § 4.1. A further restriction is that the DNA must be supported on an electrically conducting surface to define its electrical potential and therefore incident electron energy, and also to enable charge collection during the sample exposure. The plasmid used was pBR322 DNA, purified from *E. coli* strain HB101 (kindly provided by Professor R. C. Fahey, San Diego, CA, USA) using the alkaline lysis procedure as described by Sambrook *et al.* (1989). The plasmid was always >90% supercoiled as measured by gel-electrophoresis. A solution containing the DNA was spread thinly over the electrically conducting substrate and then placed in the irradiation chamber. The chamber was evacuated for 4 min to remove the water from the sample. When the pressure was $< 10^{-4}$ mbar, the sample was irradiated. It is known that the conformation of DNA changes as it is dehydrated and that this affects the rigidity of the DNA (Swarts *et al.* 1992). The implications of this are not discussed in this initial study, but will be considered later. The choice and surface finish of the substrate was found to greatly affect the spreading and drying characteristics of the DNA solution. Many materials and surface finishes were tested; the most consistent results were obtained using 25 mm diameter gold-coated ceramic discs that had been lightly sandblasted. This substrate allowed the solution to be spread thinly, whilst minimizing droplet formation during the drying process, which would cause unwanted piling up of the DNA. When viewed under an epi-illuminating microscope, the surface of the gold appears as a dense arrangement of shallow pits, each about 1 μm across. No visible difference was observed when the DNA solution was spread onto the gold and dried in the usual way. The DNA solution also contains salt (in the form of EDTA) to prevent the DNA denaturing. However, the salt is a potential source of unwanted shielding and was therefore reduced as much as possible. Various combinations of salts and salt-to-DNA ratios were tried. For the experiments described here, 0·25 μg DNA was applied in 10 μl 0·068 mmol dm^{-3} EDTA, with a salt-to-DNA ratio of 1:1 (w/w). After irradiation the DNA is returned to atmospheric conditions and rehydrated in 30 μl 10 mmol dm^{-3} Tris, 1 mmol dm^{-3} EDTA, *p*H 7·4. Recoveries of samples under these conditions were around 70%. Irradiation and recovery of the samples were done at room temperature (20°C). After irradiation the samples were stored at 4°C, before electrophoresis at room temperature. For each experiment two controls were loaded onto the gel; one was taken directly from the stock solution, while a second control was spread on the gold disc and evacuated in the usual way, but was not irradiated.

2.3. *Measurement of DNA damage*

After recovery of the DNA from the gold discs, a gel-loading buffer (40% (w/v) sucrose; 0·25% (w/v) bromophenol blue (Sigma)) was added and samples containing 0·25% μg DNA run on 1·4% (w/v) agarose gels in TBE buffer (89 mmol dm^{-3} Tris, 89 mmol dm^{-3} boric acid, 2 mmol dm^{-3} EDTA, *p*H 8·0) at 1·6 V cm^{-1} for 16 h. After electrophoresis, the gels were stained in TBE containing 0·5 μg ml^{-1} ethidium bromide, de-stained and photographed on a UV-transilluminator using Polaroid type 55 positive/negative film. The negatives were scanned with a Chromoscan 3 scanning densitometer (Joyce-Loebl Ltd) using absorbance at 530 nm. A typical profile from the densitometer is shown in Figure 2. Four forms of plasmid DNA were observed: supercoiled, relaxed, linear and, in some cases, a slow migrating band which we attributed to cross-linked DNA. In separate experiments the reading for the amount of supercoiled form was corrected for the reduced binding of ethidium bromide by comparing the fluorescence from the control DNA with identical amounts from the same sample which had been linearized by restriction endonuclease digestion. These control experiments were also carried out on serial dilutions of these samples to confirm the linear relationship between the amount of DNA and the signal from the densitometry.

3. Results

The loss of supercoiled DNA produced by electrons with incident energies between 25 and 4000 eV is shown in Figure 3, plotted against dose. The presence of a high-dose 'plateau' in the dose–effect relationship signifies that a fraction of the DNA is shielded from the electrons. The causes and implications of this are discussed in §4.1. If the unshielded DNA is converted exponentially with dose, D, then the total fraction of supercoiled DNA remaining, $f_{sc}(D)$, will be given by

$$f_{sc}(D) = f_{init}(f_{cmax}e^{-b_1 D} + 1 - f_{cmax}) \qquad (1)$$

where f_{init} is the fraction of DNA initially supercoiled (i.e. at dose, $D=0$), f_{cmax} is the maximum fraction converted (therefore $1-f_{cmax}$ is the fraction shielded), and b_1 is the conversion rate per unit dose of the DNA. This function has been fitted to the data of Figure 3 and the values of f_{init}, f_{cmax} and b_1 are given in Table 1.

The measurements of the production of linear DNA are shown in Figure 4 and have been fitted with a model based on that used by Hempel and Mildenberger (1987) to describe the production of linear DNA by ^{60}Co γ-rays. By assuming that the number of dsbs on each molecule (after receiving a dose, D) are approximated by a Poisson distribution and that more than one dsb on a molecule causes the DNA to fragment into two or more pieces (which are not scored as linear DNA), the following expression for the production of linear DNA, $f_{lin}(D)$, is obtained;

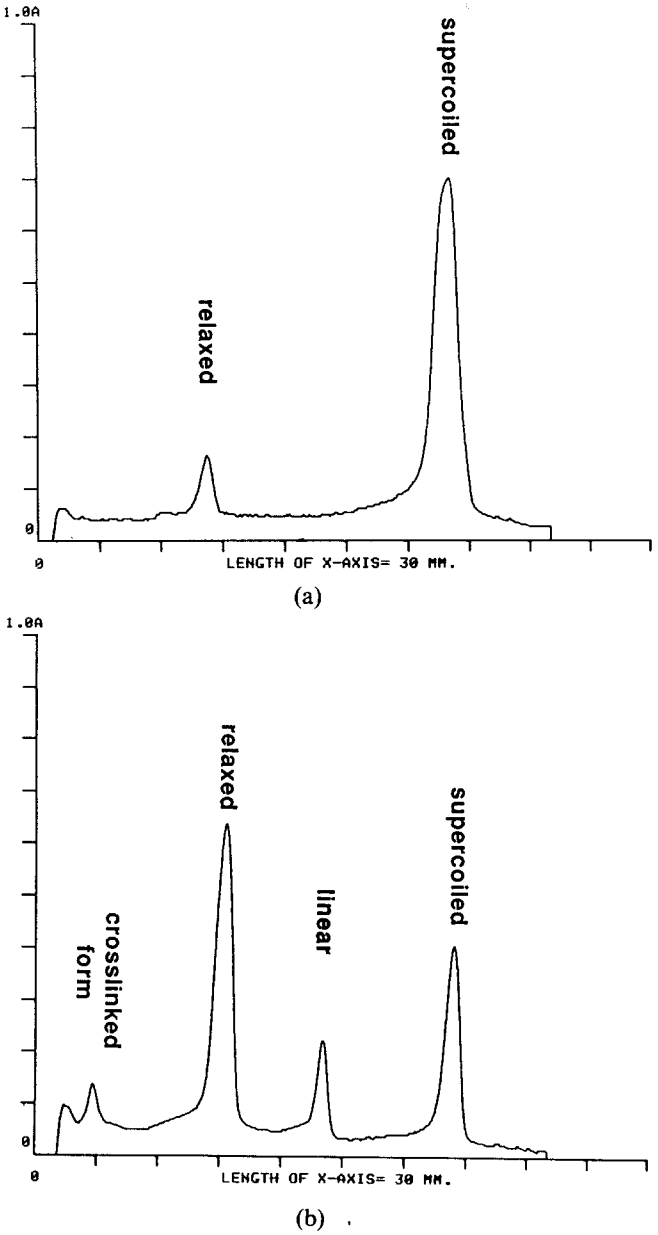

Figure 2. Sample densitometer profiles of scanned photographic negatives, prior to background correction and peak assigning, from (a) control plasmid DNA and (b) plasmid DNA irradiated with 10 000 Gy of 500 eV electrons. Ordinate-optical density, abitrary units.

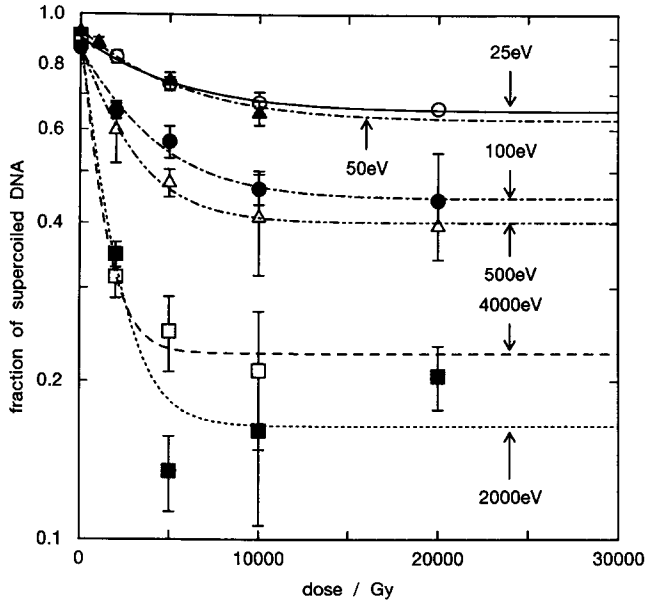

Figure 3. The fraction of supercoiled DNA remaining as a function of dose after irradiation by electrons with energies of 25 (○), 50 (▲), 100 (●), 500 (△), 2000 (■) and 4000 eV (□). The doses are at the surface of the DNA where the electrons are incident. The error bars are ±1 SE from four or more independent experiments. The curves are unweighted least-squares fits to the data. The fitted parameters are given in Table 1.

$$f_{\mathrm{lin}}(D) = f_{\mathrm{lmax}} b_2 D e^{-b_2 D}, \qquad (2)$$

where b_2 is the dsb production efficiency and f_{lmax} is the fraction of DNA that can be converted to linear and fragmented forms. Note that unlike $1-f_{\mathrm{cmax}}$, the quantity $1-f_{\mathrm{lmax}}$ is not simply a measure of the fraction shielded, but instead measures the fraction shielded or crosslinked. Note also that an explicit measurement of the fraction of fragmented DNA (which is smeared on the gel and therefore difficult to ascertain) is not necessary to establish f_{lmax} or b_2 and therefore $f_{\mathrm{lin}}(D)$. The amount of fragmented DNA can however, be estimated if necessary (Hempel and Mildenberger 1987). The values of f_{lmax} and b_2 are listed in Table 1. The maximum amount of linear DNA produced will be 37% of the value of f_{lmax}.

4. Discussion

4.1. *Shielding of the DNA*

There are several possible causes for the shielding; it may be due to the presence of overlying salt, as was suggested by Sontag and Dertinger (1975), or to the pile-up of DNA. It is also likely that, despite the evacuation procedure, some water may remain bound to the DNA molecule. It is known that up to 10% of the weight of vacuum desiccated 'dry' DNA is tightly bound water attached to the sodium phosphate group (Swarts *et al.* 1992). We were able to infer that the salt was having a shielding effect by measuring the amount of shielding for different salt concentrations. When the ratio of salt to DNA was increased, the fraction of supercoiled DNA converted to other forms and the amount of linear DNA produced both decreased. Increasing the ratio of salt to DNA from 1:1 (w/w) to 10:1 increased the fraction shielded from 18 to 45% when irradiated by 2 keV electrons. It was not possible to reduce the amount of salt present below the 1:1 ratio because the evacuating process then appeared to damage the DNA.

Indirect evidence that the DNA was overlying or piled-up is implied by the existence of slow migrating bands in the gels suggesting that crosslinked DNA is produced after irradiation. It has been shown that crosslinking occurs readily in the absence of oxygen when 'dry' DNA is irradiated with electrons (Lett and Alexander 1961). It is presumably more likely for crosslinks to form if the DNA is clumped, rather than spread thinly over the substrate. We found that the fraction of crosslinked DNA, f_{xl}, produced at high doses was as much as 20% for electrons with energies of $\geqslant$500 eV. However, almost no crosslinking was observed for 25 and 50 eV electrons and about 5% crosslinked DNA for 100 eV electrons.

An indication of how the DNA is dispersed on the substrate can be obtained by comparing the fractions of DNA not shielded, f_{cmax}, with the corresponding electron ranges (Table 1). We observe that the fraction of DNA hit increases slowly with increasing energy despite a much larger proportionate increase in the electron range. This implies that there is a distribution of shielding thicknesses across the prepared sample, from a condition of no shielding, up to as much as several hundred nanometres shielding.

By comparing the values of f_{lmax} and f_{cmax} we see that the maximum fraction of dsb-initiated damage, f_{lmax}, (i.e. linear and fragmented DNA) is always substantially less than the total fraction of DNA converted at high doses, f_{cmax}. For electron energies of $\geqslant$500 eV the fraction of crosslinked DNA produced, f_{xl}, accounts reasonably well for this difference (i.e. $f_{\mathrm{lmax}}+f_{\mathrm{xl}}=f_{\mathrm{cmax}}$). Below 500 eV very little crosslinked DNA is detected, therefore both f_{lmax} and f_{cmax} should simply be a measure of the fraction of DNA not shielded. It is surprising therefore that f_{cmax} and f_{lmax} are not the same. This disparity may reflect a real difference in the fraction of DNA

Table 1. Values of the fitted parameters from the curves fitted to the data of Figures 3 and 4 (equations 1 and 2 in the text). The errors are ±1 SE, from the least-squares fits to the data. The extrapolated electron ranges are from Iskef *et al.* (1983), corrected for density (assuming a DNA density of 1·4 g cm^{-3})

Electron energy (eV)	Electron range (nm)	$f_{init}(f_{cmax}\exp(-b_1D)+1-f_{cmax})$			$f_{lmax}b_2D\exp(-b_2D)$		
		f_{init}	f_{cmax}	$b_1 \times 10^{-5}$ Gy^{-1}	f_{lmax}	$b_2 \times 10^{-5}$ Gy^{-1}	b_1/b_2
25	0·6	0·90±0·01	0·28±0·01	20·6±3·1	—	—	—
50	1·2	0·93±0·01	0·35±0·02	17·9±2·6	0·04±0·01	9·19±7·0	2·0
100	2·4	0·85±0·03	0·48±0·03	28·5±5·9	0·12±0·02	5·55±1·9	5·1
500	14·0	0·87±0·01	0·64±0·01	40·4±3·5	0·32±0·03	8·49±1·3	4·8
2000	111·0	0·89±0·05	0·82±0·03	72·3±15·8	0·62±0·03	7·50±0·8	9·6
4000	314·0	0·91±0·02	0·75±0·02	100·0±17·0	0·60±0·02	8·55±0·6	11·7

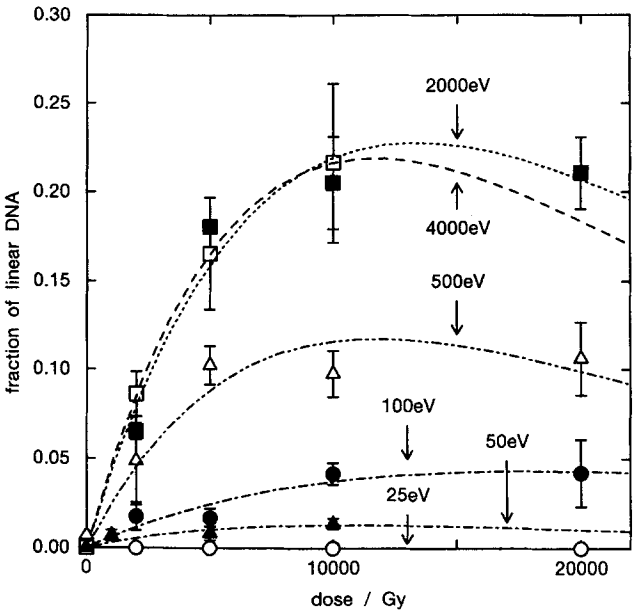

Figure 4. The fraction of linear DNA produced as a function of dose. See Figure 2 legend for key to symbols.

shielded from ssb induction compared with dsb induction, which would arise, for example, if the last few tens of eV (i.e. the last 1–2 nm of the electron track) were able to induce ssb, but not dsb. Clearly, such a disparity would predominate at low energies, when the overall electron range is very short.

4.2. *Modelling the data*

The equations describing the loss of supercoiled DNA and the production of linear DNA represent a simple treatment of the real physical conditions and processes that exist during an experiment. The simplifying assumptions are made that the electrons are monoenergetic throughout the sample, that the estimated surface dose reasonably represents the true dose, that the formation of crosslinks is independent of dose and that dsb induction is not due to two independent electrons. The discrepancies between the data of Figure 4 and the corresponding curve-fits in some cases probably reflect these simplifications. Note, however, that for the data that is of particular interest (i.e. ≤100 eV electrons), little, or no cross-linking is observed and the induction of dsbs can be shown to be due predominately to single electron interactions (see below). It is considered neither feasible nor necessary to construct a more complex model in order to obtain qualitative conclusions regarding the threshold energies for strand-break induction. The model serves primarily to show that the observed dependence on dose and energy can be understood in terms of DNA damage and shielding effects and as a means of extracting energy and dose-dependent trends from the data, and not as an attempt to derive precise quantitative information. The results shown in Table 1, and some of the analysis that follows, should be viewed in this context; the real uncertainties associated with the fitted parameters are almost certainly larger than those given, which are simply the standard errors from the curve-fits.

4.3. *DNA ssb and dsb induction*

From the values obtained for b_1, we observe that >50 eV the efficiency to convert supercoiled DNA (predominantly due to the production of ssb) increases with increasing electron energy. This is broadly consistent with the theoretical finding that the absolute number of small energy depositions within the DNA, per unit dose (of the type that can cause a ssb, but not a dsb) increases with increasing incident electron energy (Nikjoo *et al.* 1991). Electrons with the lowest energies (25 and 50 eV) have similar efficiencies at converting initially supercoiled DNA, but only 50 eV electrons appear to produce

detectable levels of DNA in linear form. The existence of 1·5% linear DNA produced by 10 kGy of 50 eV electrons is readily quantified by densitometry measurements and is clearly seen when viewing the transilluminated negative of the gel photograph. No trace of linear DNA is observed, however, after exposure to 25 eV electrons at all doses. If linear DNA is produced by 25 eV electrons, then it is with a greatly reduced efficiency compared with 50 eV electrons. We deduce from this that 25 eV electrons are apparently able to induce DNA ssb, but not DNA dsb. The observed production of ssb by 25 eV electrons is in agreement with the calculations of Charlton and Humm (1988), whose model for DNA strand-breakage successfully simulates the experimental measurements of Martin and Haseltine (1981) if a threshold energy of 17·5 eV for ssb induction is chosen.

If we assume that the linear DNA produced by 50 eV electrons is due to dsbs caused by single electron events (i.e. not due to the accumulation of damage by two or more electrons), we can conclude that a single energy deposition of no more than 50 eV within the DNA is sufficient to induce a dsb. There are several reasons to suppose that the induced dsbs are predominately due to single electron interactions; first, although the doses used are large, the probability of two electron tracks being close enough to cause a dsb is still small. For example, 50 eV electrons produce about $1{\cdot}8 \times 10^{-4}$ ssb per Gy per plasmid (i.e. the value of b_1). Therefore, a dose of 10 kGy will produce about two independent ssb per plasmid. Now a single pBR322 plasmid comprises 4363 nucleotide pairs (np), therefore assuming that a dsb can be produced by two independent ssbs on opposite strands and within 10 np of each other, then the probability of this occurrence will be 20/8726, i.e. 0·23% (after a dose of 10 kGy). This is about 10 times lower than the observed level of dsb damage (i.e. linear and fragmented DNA) by 50 eV electrons. A second reason for supposing that dsb induction is a single-electron effect is that we see no conversion to the linear form when the DNA is irradiated by 25 eV electrons, even when very large doses are used. Finally, if the conversion to linear DNA were due only to two (or more) electron events, then the dose–effect curve for linear production would have a zero initial slope. The initial slope of the 50 eV data appears to be non-zero, since a dose of only 1 kGy produces a clearly detectable fraction of linear DNA. The probability of two-electron dsb induction at this dose is very small. For higher electron energies, the initial slope is clearly non-zero.

The ratio of efficiencies of ssb and dsb production, as measured by b_1/b_2 are shown in Table 1. The ratios for the 2 and 4 keV data are similar to the theoretical calculations of Charlton *et al.* (1989) who derive an ssb:dsb ratio of about 10:1 for electrons with an energy of a few keV and are also consistent with our earlier data (Prise *et al.* 1989) for the measured induction of ssb and dsb by 1·5 keV X-rays (an ssb:dsb ratio of about 8:1 was obtained). The trend of decreasing ssb:dsb ratio with decreasing electron energy is also seen in the work of Charlton *et al.* (1989). They calculate a ratio between 5:1 and 7:1 (depending on the criterion for a dsb) for 280 eV electrons which is similar to our measured ratios for the 100 and 500 eV data (5·1 and 4·8, respectively). The ratio obtained for 50 eV electrons does seem unreasonable ($b_1/b_2 = 2{\cdot}0$). This however, may simply be a repercussion of the large uncertainty associated with b_2 in this instance.

5. Conclusions

We have been able to measure the induction of DNA breaks by electrons with energies as low as 25 eV, despite the considerable practical difficulties that arise from using electrons in this energy range. Our analysis of the data is an attempt to understand and explain the observed dose–response using a simple model for the induction of DNA damage when partial shielding is present. It is evident that large corrections for shielding are necessary to deduce the underlying effect. Because of this, and because of the simplifications inherent in the model, there are large uncertainties in our efforts to derive quantitative information from the data. Despite this there are several interesting indications regarding the thresholds for induction of DNA damage by low energy electrons; our data suggest that electrons with an energy of 25 eV can induce DNA ssb, but do not induce dsb and that the threshold electron energy for inducing dsb in dry DNA lies between 25 and 50 eV. In the future we hope to be able to minimize the problem of shielding by improving our sample preparation methods. We also intend to extend this investigation, covering a similar energy range, by exploiting the greater penetration of photons, thereby decreasing the unwanted shielding effects and introducing the possibility of irradiating the DNA in a hydrated state.

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