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Action spectra for single- and double-strand break induction in plasmid DNA: studies using synchrotron radiation

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Abstract. Ionizing radiations deposit a wide range of energies in and around DNA and this leads to a corresponding spectrum of complexity of the lesions induced. The relationships between the amount of energy deposited and the yields and types of damage induced are important in modelling the physical and chemical stages of radiation effect and linking them to biological outcome. To study these relationships experimentally, plasmids were mounted as a monolayer and exposed in vacuum to near-monoenergetic photons from the Daresbury Synchrotron. After irradiation, the DNA was washed off and assayed for single-(ssb) and double-strand breaks (dsb) using agarose gel electrophoresis. Dose–effect relationships for ssb and dsb induction were obtained at various energies in the range 8–25 eV. The initial responses in the low-dose region allowed damage yields to be estimated. However, a common feature is that the responses showed energy-dependent plateaus at higher doses as if a fraction of the DNA were shielded. Various measures were taken both to minimize and to correct for this effect. The data appear to show that the yields of ssb and dsb increase only slowly with photon energies >10 eV, with a suggestion of similar threshold energies for both lesions. In the energy range covered, the yield of ssb is 12–20-fold greater than that of dsb. The data indicate that ssb and dsb may have a common precursor in this system. Earlier work with low-energy electrons showed that at 25 eV ssb were induced but no dsb were detected.

1. Introduction

Energy loss studies have shown that the most frequent energy depositions by high-energy ionizing radiations are on the order of tens of electron volts in several organic materials (Rauth and Simpson 1964) and in nucleic acid (Johnson and Rymer 1967). Monte Carlo simulations of the interactions of charged particle tracks with DNA have allowed the spectra of energy depositions to be predicted for various types of radiation (Charlton *et al.* 1989, Goodhead and Nikjoo 1989). However, little experimental information exists about the relationships between energy deposition size and the types, yields and complexity of the damaged sites induced. Lücke-Huhle and Jung (1973a,b) used metastably excited gases from 4·3 to 19·8 eV to induce damage in dry ΦX174 DNA, but found that the actions were generally different from those of ionizing radiations. Wirths and Jung (1972) and Sontag and Dertinger (1975) used vacuum UV light (VUV) to study inactivation and single-strand breakage (ssb) in dry ΦX174 DNA. More recently, studies by Bothe *et al.* (1990) and by Gurzadyan and Görner (1993) have used pulsed laser light at ~5 or ~6 eV to study ssb and double-strand break (dsb) induction in aqueous solutions of calf thymus or plasmid DNA. Hieda (1994) has measured ssb and dsb induction in pBR322 plasmid DNA by photons from 8 to 2 000 eV. We have previously used low-energy electrons to measure the energy dependences for ssb and dsb induction in dry pBR322 plasmid DNA (Folkard *et al.* 1993). This paper reports our initial data, also with plasmid DNA, obtained using VUV from a synchrotron radiation source at selected energies in the range 8–25 eV, determining action spectra for ssb and dsb induction.

2. Methods

2.1. *Vacuum UV irradiation*

Irradiations were carried out at station 3.1 (dipole magnet) of the 2-GeV Daresbury electron synchrotron (DRAL Daresbury Laboratory, Daresbury, UK). Photon energies in the range 8–25 eV were selected using a Seya-Namioka mount 1 m grating monochromator. Higher-order diffracted light was filtered out using windows of LiF, In:Ti or Al:Si, as appropriate. Details of the irradiation arrangement and dosimetry will be given in a future publication.

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DNA samples were evacuated to $<10^{-5}$ mbar before starting exposure. Unirradiated control samples were placed in a section which was not exposed to the beam but was subjected to exactly the same evacuation cycle as the irradiated samples. After irradiation, the sample chamber was refilled with nitrogen before removing the control and irradiated samples to atmosphere.

2.2. *Sample preparation and assay*

pBR322 plasmids (4363 bp) were used as described previously (Folkard *et al.* 1993). In general, the plasmids as initially prepared were >90% in the supercoiled form, although some degradation was introduced by the handling procedures (see below). The aim was to produce a dry monolayer of plasmids for exposure to the VUV beam. In our initial work this was done by placing a 10-μl drop of solution containing 0·25 μg DNA and 0·025 μg salt (in the form of EDTA) on to a 13 mm diameter polycarbonate filter (10-nm pore size, Poretics Corp., Livermore, CA, USA) and spreading continuously over an area about 9 mm in diameter while drying took place to atmosphere. This was similar to the procedure used previously (Folkard *et al.* 1993) and, as before, the shapes of the dose–effect curves were indicative of a partially shielded response due to failure to achieve the desired uniform monolayer of DNA and EDTA. The methods used to rehydrate and recover the samples after exposure and to assay for damage using gel electrophoresis have been described previously (Folkard *et al.* 1993).

3. Results

The loss of the supercoiled fraction of pBR322 plasmids as a function of exposure to 25 eV photons is shown in Figure 1a. Loss of the supercoiled form is mainly due to conversion to the relaxed circular form by ssb induction. The proportion of conversion to the linear form by dsb induction is relatively small. Control samples were placed in a side compartment of the irradiation chamber vacuum at the same time as the irradiated samples, but were shielded from the VUV light. They showed no significant increase in conversion with the duration of the exposure, indicating that prolonged evacuation does not cause appreciable damage. However, the proportion of the supercoiled form in the control samples was 10–20% lower than that of the stock solution, and was consistent with a constant small amount of ssb induction due to the handling and evacuation procedures. A straight line has been fitted to the data for the exposed samples in Figure 1a indicating a reasonable fit compatible with the conversion being exponential with VUV exposure. However, with these and other data there is a strain to the fit suggesting that underlying response is curved as if the sensitivity decreases with increasing exposure. Similar behaviour was found in our earlier study with low-energy electrons (Folkard *et al.* 1993) and in other work with low-energy photons (e.g. Wirths and Jung 1972, Sontag and Dertinger 1975) and has been attributed to partial shielding effects. Where appropriate, allowance has been made, as in our electron work, and a value derived for the responsiveness in the low-dose region.

Conversion to the linear form is shown in Figure 1b and represents the induction of dsb in the supercoiled form and, increasingly at the higher doses, in the potentially relaxed circular form. The data for the control samples (not shown) show that the amount of conversion due to the handling and

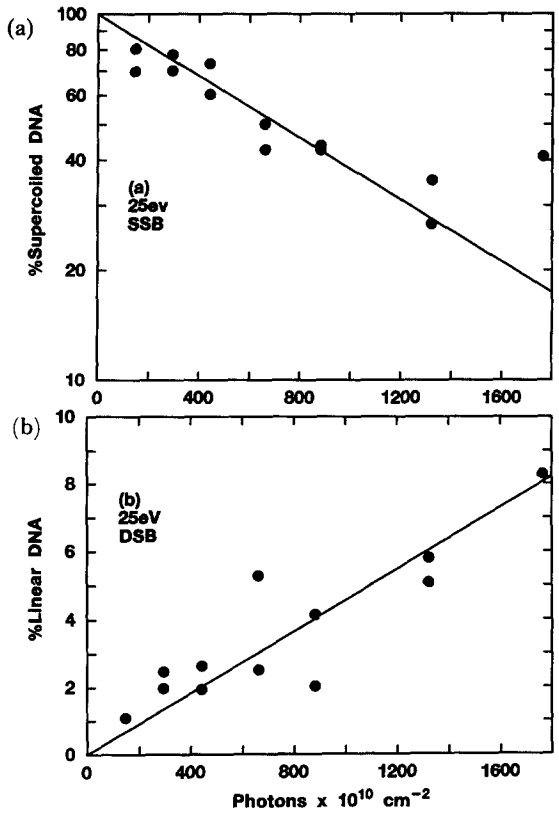

Figure 1. Loss of supercoiled (a) and formation of linear (b) pBR322 plasmids versus exposure to 25 eV photons. The values of supercoiled and linear produced have been corrected for the % supercoiled and linear values measured in control samples exposed to the vacuum conditions at the same time.

evacuation procedures is very small. A straight line has been fitted to the data and these and our other data suggest a downward curved response would give a better fit. Theoretically, an expression of the form $\alpha D.\exp(-\alpha D)$ should apply (Hempel and Mildenberger 1987), but for the range of conversion to linear shown this would account for only very slight downwards curvature. As for ssb induction, the data for dsb therefore also suggest the occurrence of partial shielding.

Figure 2 shows the yields of ssb and dsb calculated from the dose–response curves obtained from 8 to 25 eV using pBR322 plasmids on polycarbonate filters, dried under atmospheric conditions. The yields are expressed as breaks per plasmid per incident photon.

4. Discussion

Earlier work had shown induction of ssb in dry ssb (Sontag and Dertinger 1975) and dsb (Wirths and Jung 1972) ΦX174 DNA at photon energies of 4·9 eV, the efficiency rising steeply up to ~11 eV and thereafter changing little up to 21·2 eV, the maximum energy used. Ito (1992) has reported ssb induction in pBR322 plasmids from ~5 to ~15 eV. Our data for ssb induction in pBR322 plasmids show similar features, but the yield falls less steeply below ~11 eV. We also report data for dsb induction, and it is interesting to note that the shape of the action spectrum is similar to that for ssb induction, but the yield is 12–20-fold lower. The data suggest that, in this dry DNA system, 8–25 eV photons induce ssb and dsb via a common precursor species, which has a 12–20-fold higher probability of generating a ssb than a dsb. Another explanation for similar action spectra for ssb and dsb formation would be if dsb arose from pairs of ssb separately induced on opposite strands. Two factors argue against this. This first is that the dose–effect data for dsb show no evidence of the positive quadratic term (i.e. an upwards curvature in Figure 1b) that would be required for a two-event induction. The second is that the exposure levels used in this study induced at the most an average of a few ssb per plasmid and the probability of any two ssb arising on opposite strands, and within a sufficiently short distance to form a dsb, would therefore be rather small. Other studies have proposed the existence of a single-event pathway to dsb induction (Boon *et al.* 1984, Siddiqi and Bothe 1987, Krisch *et al.* 1991), but it has also been shown that multiple-event induction may occur (Ward 1981, Krisch *et al.* 1991, Prise *et al.* 1993). Bothe *et al.* (1990) have shown ssb and dsb induction in aqueous solutions of calf thymus DNA by laser pulses at 248 nm (~5 eV). Gurzadyan and Görner (1993) have reported these and other forms of damage in aqueous solutions of plasmid and calf thymus DNA by laser pulses at 193 nm (~6 eV).

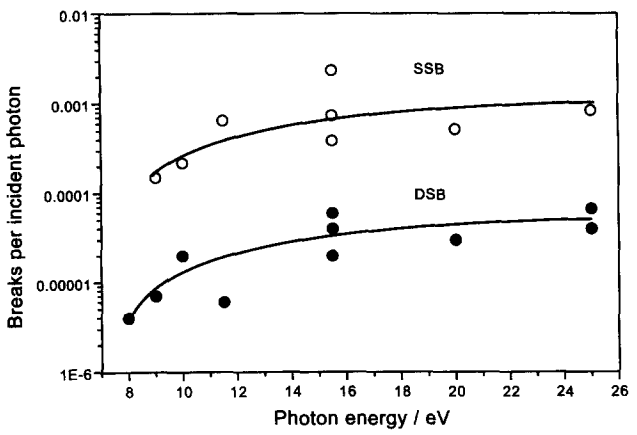

Figure 2. Yields of ssb (○) and dsb (●) in pBR322 plasmids versus photon energy.

An alternative possibility is that dsb arise from ssb induced opposite single-strand damage caused during the handling procedures. Unirradiated samples show 20–30% conversion to the relaxed form, corresponding to a mean of ~0·3 ssb per plasmid due to the handling procedures alone. The maximum probability of these 'handling' ssb combining randomly with a radiation-induced ssb to form a dsb would be $0{\cdot}3 \times 0{\cdot}5 = 0{\cdot}15$, allowing a 0·5 probability of the breaks being on opposite strands and assuming that a dsb forms even if the breaks are the furthest distance apart, 2181 bp. This would correspond to a ssb:dsb ratio of ~7:1, i.e. about half of what we observe. Thus, to explain the observed ssb:dsb ratio by this mechanism alone would require cooperation of ssb up to ~1000 bp apart, which is much greater than other studies have indicated. In our earlier work using low-energy electrons to irradiate pBR322 under vacuum (Folkard *et al.*1993), in which at 25 eV we observed ssb but no detectable dsb, essentially the same handling conditions were used; this finding would, therefore, also argue against the involvement of 'handling' ssb in dsb production and, similarly, against long-range cooperation of ssb to form dsb in this dry plasmid system. We therefore consider that this mechanism of dsb induction is unlikely to explain more than a small fraction of the observed yield of dsb.

In our earlier work (Folkard *et al.* 1993), exposure to 25 eV electrons gave efficient induction of ssb but no detectable dsb. At an electron energy of 50 eV, ssb and dsb were induced. Thus the pattern of damage

formed appears to depend not just on the amount of energy deposited but on the nature of the energy transfer. Modelling studies by Charlton *et al.* (1989) indicate a threshold energy for induction of ssb at 17·5 eV, which is higher than indicated by these and the earlier VUV data. Their model indicates that dsb are not induced by energies below about 40 eV, which is in closer accordance with our earlier observations for electrons than with the present data for photons.

5. Conclusions

This and our earlier study (Folkard *et al.* 1993) show that considerable information can be gained about damage induction processes using low-energy radiations. The availability of synchrotron, and also excimer laser, sources offers opportunities to examine the effects of energy transfers selectively within the broad spectrum that occurs when energetic ionizing radiations traverse DNA. As well as being of mechanistic interest, the data so obtained may assist with mathematical modelling of radiation interactions and effects. Although our studies have so far concentrated on direct effects in dry plasmids, a necessary future development will be to extend the studies to hydrated samples.

Acknowledgements

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