Effects of Hydration on the Induction of Strand Breaks, Base Lesions, and Clustered Damage in DNA Films by α-Radiation

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The yields of DNA single (ssb)- and double-strand breaks (dsb) as well as base lesions, which are converted into detectable ssb by base excision repair enzymes, induced at 278 K by densely ionizing α-radiation have been determined as a function of the level of hydration (Γ, number of water molecules per nucleotide) of films of supercoiled plasmid DNA (pUC18). The yields of prompt ssb induced by α-radiation are independent of Γ, from vacuum-dried up to 35 water molecules per nucleotide, indicating that diffusible hydroxyl radicals or $H_2O^{\bullet+}$, if induced in the hydrated layer by α-radiation, do not significantly contribute to the induction of ssb. In contrast, the yield of prompt dsb does increase with increasing hydration level. At a Γ of 35, the yield of dsb is about twice that for γ-irradiation. Treatment of α-particle-irradiated DNA with the enzymatic probes, endonuclease III (Nth), and formamidopyrimidine-DNA glycosylase (Fpg), does not lead to significant levels of additional ssb and dsb. It is proposed that (i) base lesions induced by direct energy deposition in the DNA−water complex by high LET radiation, such as α-particles, are generally present in clustered DNA damage, e.g., two or more lesions produced within a few tens of base pairs, and (ii) the complexity of the clustered damage, lesion density, is greater for densely (compared with sparsely) ionizing radiation. As a consequence, the majority of DNA base lesions induced by high LET radiation, in contrast to those by low LET radiation, and which are substrates for Fpg and Nth become refractory to excision repair due to their formation within more complex, clustered DNA damage.

Introduction

Many studies on high linear energy transfer (LET) ionizing radiation, e.g., α-radiation, have highlighted its severe biological effects relative to that of low LET radiation such as X-rays (see ref 1 for review). The biological effects of ionizing radiation are thought to arise from the formation of clustered DNA damage, e.g., two or more lesions (base lesion, single strand breaks (ssb), abasic site) formed within about 10 base pairs separation by a single radiation track. Earlier studies have concentrated on the relation between the LET of radiation and the efficiency of induction of DNA double strand breaks (dsb) (see ref 2 for review), the repairability of DNA dsb,[3] the formation of short DNA fragments in living cells[4−7] and, using isolated DNA,[8−11] the dependence of the yield of DNA strand breaks on radiation quality. More recent studies have shown that clustered DNA damage is formed in cells by both densely ionizing radiation[12] and sparsely ionizing X- or γ-ray radiation,[13−15] as predicted from DNA damage simulations,[16] whereby DNA base lesions are often associated with other lesions particularly with high LET radiation such as α-particles. Most mechanistic studies[17−19] on DNA damage induction to date have focused on the indirect effects of radiation by which DNA damage is induced by diffusible water radicals, whereas much less is known about the mechanisms of the direct effects of radiation,[19−22] which also lead to persistent DNA damage. Recently we have shown[20] that the induction of base lesions and clustered DNA damage by direct energy deposition in hydrated DNA by γ-radiation arises from not only ionization of the bases but also from their oxidation by $H_2O^{\bullet+}$ in competition[23,24] with its conversion into an hydroxyl radical. This latter pathway is important in understanding the processes which lead to radiation degradation of DNA in cells or biological samples.

The present study investigates the induction of clustered DNA damage in hydrated plasmid DNA using densely ionizing α-particles. Knowledge of the processes resulting in radiation degradation of DNA by direct effects of α-radiation will greatly increase understanding of the deleterious biological effects of ionizing radiation at the molecular level. For instance, ∼30−40% of the lesions induced in cellular DNA by low LET radiation arise from direct energy deposition events (direct effects) with this value increasing to >70% for α-particle radiation.[25] From biophysical simulations, the proportion of radiation induced base lesions which are present in clustered DNA damage is significant, ∼20% for low LET radiation rising to ∼70% for α-radiation.[16] Recently, the use of enzymatic probes has provided valuable insight into the yields of base lesions induced in plasmid DNA in solution by systematically changing radical scavenger concentration[11,26−28] or in hydrated DNA samples[20] using either α- or γ-radiation. The latter study provided evidence that the yield of γ-radiation-induced, non-dsb containing clustered DNA damage, visualized as additional dsb by treatment with base excision repair enzymes, increases with increasing the level of hydration.

In this paper we provide the first report on the direct effects of densely ionizing α-radiation on the induction of damage in

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hydrated plasmid DNA as revealed by enzymatic probes (base excision repair proteins endonuclease III (Nth) and formamidopyrimidine-DNA glycosylase (Fpg)). Of particular importance is assessing the influence of the ionization density of the radiation on the yields of biologically relevant clustered DNA damage through comparison with our previous data[20] obtained with sparsely ionizing γ-radiation.

Materials and Methods

Plasmid DNA Preparation. Plasmid DNA (pUC18, 2686 base pairs) was obtained from an *Escherichia coli* HB101 host and extracted using alkali-lysis, followed by purification with double banding on cesium chloride−ethidium bromide gradients as described previously.[29] The plasmid, which is over 90% in the closed circular form, was subsequently stored at −20 °C in TE buffer (10 mmol dm^{-3} Tris, 1 mmol dm^{-3} EDTA, pH 8.0) at a concentration of 2.2 mg/mL.

Humidity Control of the DNA Samples. The control of the humidity of DNA samples is as described previously.[20] Briefly, the stock solution of DNA was diluted with TE buffer to give a final DNA concentration of 0.11 mg/mL. Aliquots (5 μL) of this plasmid solution at 4 °C were spotted onto a Hostaphan base of a titanium walled irradiation dish and dried by blowing dry air (less than 10% humidity) for 30 min at 4 °C to avoid crystallization of the buffer solutes by a flash-freeze-drying procedure. After drying, a uniform film of DNA/buffer solutes of 3 mm diameter was formed on the Hostaphan base of the dish, which was then placed in a freeze-drying apparatus (Micro-Modulin, Edwards) for 1 h. Twelve plasmid DNA films were normally prepared on a single dish.

After freeze-drying, the DNA films were hydrated using two concentrations of sodium hydroxide of 6.56 and 3.84 mol dm^{-3} to give relative humidities of 83 and 97%, respectively. The relative humidity within the unsealed dish within a plastic chamber was measured using a hygrometer (Hygrotest 6400, Testoterm Ltd.) inserted in the chamber. The humidity control chamber was maintained at 5.7 ± 0.5 °C for >15 h to equilibrate the samples[20] prior to α-particle irradiation. Under these conditions of humidity control, <5% degradation of the closed circular DNA occurs. At 97% humidity, the thickness of the samples is <3 μm, determined by laser scanning confocal microscopy,[20] and does not significantly affect the LET of the α-particles.

For vacuum-dried conditions, plasmid DNA on the Hostaphan base of a dish was irradiated with α-particles immediately after the freeze-drying procedure. During irradiation, the sample chamber was kept at <15% humidity by filling the chamber with dry air. It was assumed that exposure to dry air does not significantly affect the level of hydration of DNA, since the irradiation time (maximum 3 h) is short compared with that to attain the required humidity (15 h) as shown previously.[20,26]

α-Particle Irradiation of the DNA Samples. Following establishment of the required humidity, the irradiation dish (see Figure 1) was sealed and then placed on the α-particle irradiator, which has been described previously.[3,30] The container labeled **1** in Figure 1 contained NaOH solution of the required concentration to maintain the humidity of the samples during irradiation. The temperature during irradiations was controlled at 5.7 ± 0.5 °C using a chiller thermocirculator (Churchill Instruments Co. Ltd.) pumping a water/antifreeze mixture around a jacket surrounding the dish. The α-particle spectrum has a peak energy[3] of 3.31 MeV. The absorbed dose rate, determined by flux measurements using CR-39 plastic track detectors,[31] was 20 Gy/min. The maximum irradiation time 3.75 h delivers a dose of 4.5 kGy. After α-particle irradiation, the sample was recovered with 10 μL of TE buffer (4 °C) and stored at −20 °C (for maximum 4 h), prior to determination of the yield of prompt strand breaks by agarose gel electrophoresis. If the irradiated samples and unirradiated control were to be subsequently treated with enzymes, 5 μL of sodium acetate (1 mmol dm^{-3}) and 30 μL of chilled ethanol were subsequently added. Following DNA precipitation and centrifugation as described previously,[20] the samples were then treated as described below.

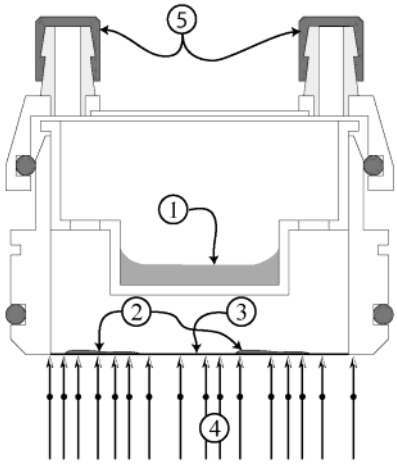

Figure 1. Cross-section of the irradiation dish where **1** labels the hydration solution, **2** the DNA film, **3** the Hostaphan dish base, **4** the α-particle beam, and **5** gas port seals.

Detection of Enzyme-Sensitive DNA Damage. Purified proteins, Nth and Fpg, were generous gifts from Prof. Rick Wood and Dr. Roldan-Arjona (Imperial Cancer Research Fund). Stock solutions (50% glycerol, 100 mmol dm^{-3} potassium phosphate, 0.1 mol dm^{-3} diethioethanol (DTT), and 0.005% Triton X-100)[32] of either Nth or Fpg at a concentration of 1.3 ng/μL and 21 ng/μL respectively at pH 6.6 were stored at −20 °C.

The precipitated plasmid DNA was dissolved in 20 μL of reaction buffer (0.5 mmol dm^{-3} EDTA, 0.1 mol dm^{-3} KCl, 40 mmol dm^{-3} HEPES, 0.5 mmol dm^{-3} DTT and 0.2 mg/mL bovine serum albumin (BSA))[33] at pH 8.0. The control and irradiated samples were incubated for 30 min at 37 °C in either the presence or absence of the proteins. The incubation conditions and concentrations of Nth and Fpg of 1.1 ng and 19 ng per μg of DNA respectively were optimized previously,[20,26] as degradation of the closed circular form of the plasmid DNA was not seen. Following incubation of irradiated DNA or controls with the enzymes, 5 μL of 0.5 mol dm^{-3} EDTA was added to quench the activity of the enzymes and the solutions placed on ice prior to quantification by agarose gel electrophoresis.

The samples were categorized into three groups depending upon the post-irradiation treatment. The first group was maintained at −20 °C (termed *prompt ssb* or *prompt dsb* in the remainder of this paper), the second was incubated at 37 °C for 30 min in the absence of enzyme (*prompt+heat-labile ssb*) and the third incubated at 37 °C for 30 min in the presence of Nth, Fpg, or Nth+Fpg ("*Nth+ssb*", "*Fpg+ssb*", or "*Nth+Fpg+ssb*").

Quantification of the Yields of Strand Breaks in Irradiated Plasmid DNA. Prior to agarose gel electrophoresis, 5 μL of the loading buffer (0.1% bromophenol blue, 30% sucrose in TBE (89 mmol dm^{-3} Tris, 89 mmol dm^{-3} boric acid, 2 mmol dm^{-3} EDTA)) was added to the solutions containing irradiated or control DNA. Either 15 μL of solutions containing DNA

TABLE 1: Yields of ssb and dsb Induced in Dry PUC 18 Plasmid DNA by α-Particle Irradiation under Different Levels of Hydration (the respective errors are shown in parentheses)

treatment of sample	Γ (mol water/mol nucleotide)	$D_{37} \times 10^3$Gy	ssb/Gy/Da ($\times 10^{-11}$)	G(ssb) $\times 10^{-6}$ mol/J	dsb/Gy/Da ($\times 10^{-11}$)	ssb/ dsb
prompt damage	4 (1.5)	12.0 (1.0)	4.8(0.4)	0.048(0.004)	1.0(0.2)	4.8
	14.5 (4.1)	12.3 (1.2)	4.6(0.6)	0.046(0.006)	1.5(0.1)	3.1
	34.5 (9.7)	9.6 (1.2)	6.0(0.9)	0.060(0.009)	1.7(0.1)	3.5
prompt + heat	4 (1.5)	11.4 (0.3)	5.0(0.2)	0.050(0.001)	0.8(0.2)	6.0
labile damage (II)	14.5 (4.1)	7.7 (1.0)	7.5(1.0)	0.075(0.01)	1.4(0.1)	5.4
	34.5 (9.7)	11.3 (1.0)	5.0(0.5)	0.050(0.005)	1.3(0.1)	3.8
II + Nth	4 (1.5)	9.7 (0.6)	5.9(0.4)	0.059(0.004)	1.0(0.1)	5.9
	14.5 (4.1)	8.0 (0.4)	7.1(0.4)	0.071(0.004)	1.5(0.1)	4.7
	34.5 (9.7)	8.9 (0.9)	6.4(0.7)	0.064(0.007)	1.5(0.1)	4.3
II + Fpg	4 (1.5)	9.6 (1.0)	5.9(0.6)	0.059(0.006)	1.3 (0.1)	4.5
	14.5 (4.1)	9.8 (0.1)	5.8(0.1)	0.058(0.001)	1.6(0.1)	3.6
	34.5 (9.7)	9.4 (0.9)	6.1(0.7)	0.061(0.007)	1.6(0.1)	3.8

treated with enzyme(s) or 7.5 μL of nonenzymatically treated solutions was placed into the well of a 1 % agarose (Sigma Type 1-A) gel in TBE buffer at pH 7.1. The samples were run at typically 75 mV cm^{-1}, 7 mA for 17 h at 5.7 °C. Following electrophoresis, the gel was stained with 30 μL of ethidium bromide (10 mg/mL) in 600 mL of TBE buffer for 1 h at 5.7 °C. The separated closed circular, open circular, and linear forms of the plasmid DNA in the gel were visualized using a UV transilluminator, and an image of the gel was obtained using a charge-coupled device (CCD) camera. The relative amount of DNA in each form was then quantified as described previously.[29] The effect of superhelical density on the correction factor for ethidium binding to closed-circular DNA has been discussed previously.[29,34]

A dose response was determined from the logarithmic loss of closed circular plasmid DNA on radiation dose at the specified humidity. From the slope of this response, a D_{37} value was obtained which, assuming Poisson statistics for ssb induction, represents the radiation dose required to give on average one ssb per plasmid molecule. Using the D_{37} value, an average number of ssb/Gy/Da (n(ssb)) was obtained, assuming an average mass of a base pair of 650 Da and knowing pUC18 DNA contains 2686 base pairs.

$$\mathrm{n(ssb)} = 1/(2686 \times 650 \times D_{37}) \quad (1)$$

The average number of dsb/Gy/Da (n(dsb)) was determined from the dose dependence of the fractional abundance of the linear form of the DNA given by

$$\mathrm{n(dsb)} = b/(2686 \times 650) \quad (2)$$

where b is obtained from the slope of the dose response.

G Values for Damage. Careful consideration is needed to calculate radiation chemical yields, G values, for strand breaks in these hydrated DNA samples. For the dry/hydrated samples, it is reasonable to assume that a given α-particle flux delivers the same dose to DNA molecules for a fixed amount of DNA at the different levels of hydration. Based on this assumption, the G value (mol/J) is given by

$$\mathrm{G(ssb)} = (1000/\mathrm{mwt})/D_{37} \quad (3)$$

where mwt is the molecular weight of pUC18 plasmid DNA (1.746 M Dalton).

Results

Dependence of the Yield of ssb on the Level of Hydration. The dependence of the amount of closed circular DNA on radiation dose is shown in Figure 2 for α-particle irradiation of pUC18 plasmid DNA at 5.7 °C at a relative humidity of 97% (Γ = 34.5). Γ is defined as the number of water molecules per nucleotide. Our previous data[20] for γ-irradiated DNA is shown for comparison.

From this dependence, the yield of ssb was calculated from the D_{37} value (see eq 1). The yields of ssb were determined at different relative humidities from similar dose dependences for loss of closed circular DNA irradiated at 5.7 °C. The dependence of the yield of ssb/Gy/Da on the level of hydration (Γ) is shown in Figure 3.

The mean value of Γ with standard deviation (see Figure 2 and Table 1) at the respective relative humidity was determined from three independent relationships between relative humidity and the level of hydration of DNA reported in the literature.[35−37] It has been assumed that Γ of vacuum-dried samples is ~4 ± 1.5 under the conditions of the freeze-dry procedure.[37,38] From Table 1, the yield of *prompt ssb* induced by α-particles does not show a significant dependence on the level of hydration (Γ) over the range of hydration studied. The yield of additional

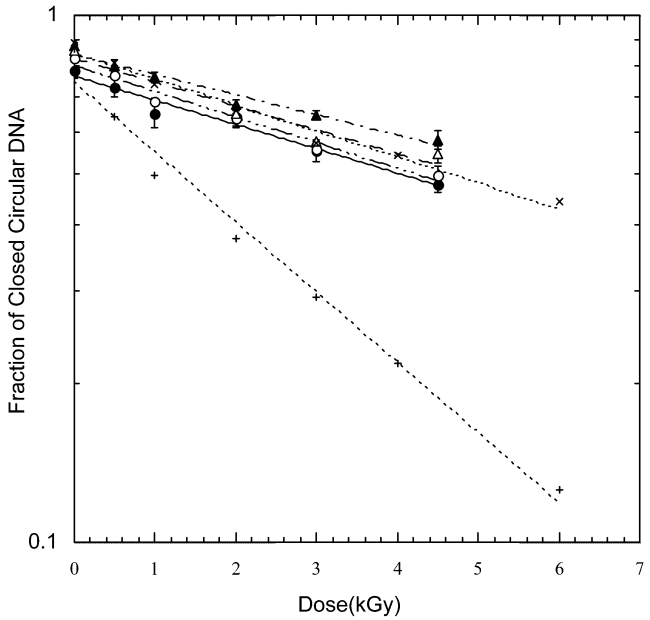

Figure 2. The dependence of the loss of closed circular DNA on radiation dose after exposure of pUC18 plasmid DNA to α-particles at 5.7 °C and a relative humidity of 97% (○) or following a post-irradiation incubated for 30 min at 37 °C in the absence (●) or presence of either Nth (▲) or Fpg (△). The points represent the mean ± SD of three independent experiments. Each line represents a least-squares fit to the data points. Our previous data[20] with γ-irradiation are shown for comparison; a post irradiation incubation for 30 min at 37 °C in the absence (×) or presence of Nth (+).

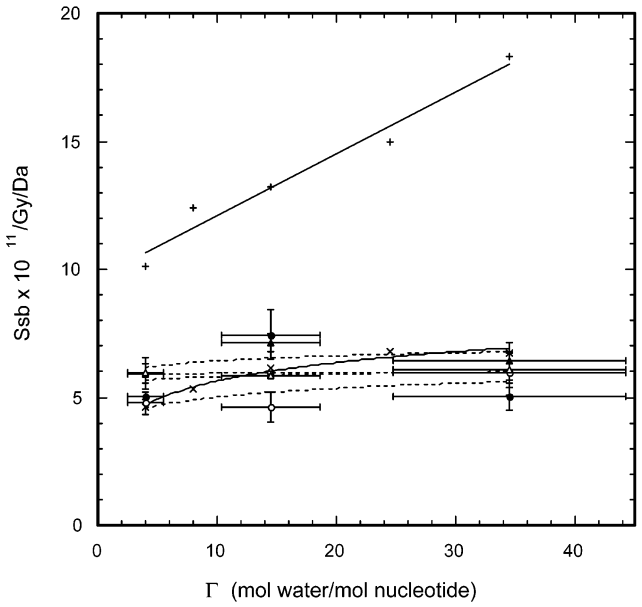

Figure 3. The dependence of the yield of ssb on Γ (see text) for DNA samples α-particle irradiated at 5.7 °C (○) or following a post-irradiation incubated at 37 °C for 30 min in the absence (●) or presence of either Nth (▲) or Fpg (△). The vertical error bars are ±SD for the values of ssb determined from the slope of the dose−response curve for three independent experiments. The horizontal bars represent the mean values of Γ with standard deviation (see Table 1). Solid lines indicate our previous data[20] with γ-irradiation shown for comparison; a post-irradiation incubation for 30 min at 37 °C in the absence (×) or presence of Nth (+). The curves have been included to guide the eye.

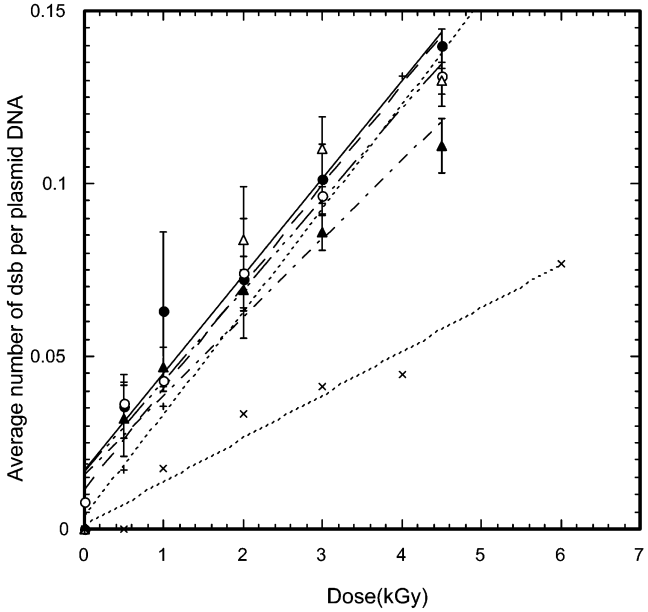

Figure 4. The dependence of the number of dsb, determined from the fraction of the linear form of DNA, on α-radiation dose after exposure of pUC18 plasmid DNA at 5.7 °C and a relative humidity of 97% (○) or following a post-irradiation incubated for 30 min at 37 °C in the absence (●) or presence of either Nth (▲) or Fpg (△). The points represent the mean ± SD of three independent experiments. Each line represents a least-squares fit to the data points. Our previous data[20] with γ-irradiation are shown for comparison; a post irradiation incubation for 30 min at 37 °C in the absence (×) or presence of Nth (+).

ssb, induced by a heat treatment, is low since the dependence of the yield of ssb/Gy/Da on Γ determined at 5.7 °C is similar to that determined after post-irradiation heat treatment at 37 °C for 30 min (Figure 2). The maximum yield of the *prompt ssb* (or the *prompt+heat-labile ssb*) is $\sim 7 \times 10^{-11}$ ssb/Gy/Da.

Yields of ssb after Nth and Fpg Treatment. Post-irradiation incubation of the plasmid DNA with either Nth or Fpg at 37 °C does not show significant additional loss of closed circular DNA for a given α-radiation dose at 97% (Γ = 34.5) humidity level, as shown in Figure 2. The yields of both *Nth+ssb* and *Fpg+ssb*, determined from dose dependences for the loss of closed circle DNA, show similar values for all levels of hydration with those obtained in the absence of an enzyme treatment, as shown in Figure 3 and compiled in Table 1.

Dependence of the Yield of dsb on the Level of Hydration. The induction of dsb by α-particle irradiation of plasmid DNA at 5.7 °C or following incubation in buffer for 30 min at 37 °C increases linearly with increasing dose as shown in Figure 4 for a relative humidity of 97% (Γ = 34.5). The yields of dsb/Gy/Da were calculated from the dose dependences using eq 2. Significant levels of additional dsb are not induced by a heat treatment, since the yields of *prompt dsb* and *prompt+heat-labile dsb* at the various levels of hydration are similar, as shown in Figure 5. An increase of the dsb yields with increasing value of Γ is seen in the lower hydration region (Γ < 15), but at higher values of Γ the yields tend to a plateau value. The maximum yield of prompt dsb at Γ = 34.5 is $\sim 1.6 \times 10^{-11}$ dsb/Gy/Da. The yields of dsb, n(dsb), at various levels of hydration are listed in Table 1, together with the ratios of n(ssb)/n(dsb).

Yields of dsb after Nth and Fpg Treatment. Incubation of the plasmid DNA with either Nth or Fpg at 37 °C post α-particle irradiation results in a linear induction of dsb on dose at 97% relative humidity (Γ = 34.5), as shown in Figure 4. The yields

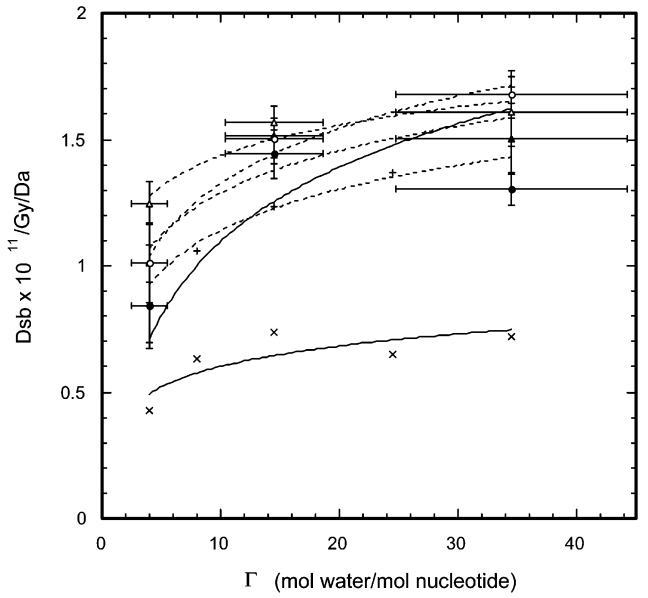

Figure 5. The dependence of the yield of dsb on Γ for DNA samples irradiated at 5.7 °C (○) or following a post-irradiation incubated at 37 °C for 30 min in the absence (●) or presence of either Nth (▲) or Fpg (△). The vertical error bars are ±SD of the slope of the dose−response curve for three independent experiments. The horizontal bars represent the mean values of Γ with standard deviation (see Table 1). Solid lines indicate our previous data[20] with γ-irradiation is shown for comparison; a post irradiation incubation for 30 min at 37 °C in the absence (×) or presence of Nth (+). The curves have been included to guide the eye.

of both *Nth+dsb* and *Fpg+dsb* induced by α-particles at all levels of hydration are similar to those in the absence of an enzyme treatment as shown in Table 1 and Figure 5. The ratios of ssb/dsb decrease slightly with increasing level of hydration (ssb/dsb∼3−6), as shown in Table 1.

Discussion

Clustered DNA damage of different complexity (number of lesions within the cluster) is more efficiently induced by densely (compared with sparsely) ionizing radiation.[39,40] Theoretical studies using a Monte Carlo simulation[16] revealed that DNA lesions, consisting of strand breaks and/or base lesions/abasic sites, are induced by an α-particle track, mainly as clustered DNA damage when the radiation track intersects a DNA molecule. Clustered DNA damage may be more efficiently produced by direct energy deposition in DNA and its hydration shell than by indirect effects involving diffusible water radicals. Although several studies focused on the effects of DNA hydration on the yields of isolated lesions[21,22,41,42] and clustered DNA damage[20] induced by sparsely ionizing radiation, less is known about the yields of damage induced by densely ionizing radiation, where the cellular contribution to DNA damage induced by direct effects predominates. In this study, the direct effects of densely ionizing α-particles on the induction of DNA strand breaks and base lesions, detected as enzyme sensitive sites (ess), were studied systematically at various hydration levels of plasmid DNA. The main findings from this study on the direct effects of α-radiation on hydrated DNA are (i) the yields of radiation-induced *prompt ssb* are independent of the level of DNA hydration, (ii) the yields of *prompt dsb* increase with hydration, (iii) heat labile sites are not produced in detectable amounts, and (iv) surprisingly ess were not revealed in detectable amounts within the range of hydration studied when α-particle irradiated DNA is treated with Nth and Fpg.

The dependences of the yields of DNA damage on hydration have been compared with those[20] from γ-irradiation. The role of Tris present in the samples and its influence as an hydroxyl radical scavenger were discussed in detail by Yokoya et al.[20] The yields of DNA *prompt ssb* induced by α-particle irradiation ($\sim 6 \times 10^{-11}$ ssb/Gy/Da) are similar or slightly lower by ~20% than those[20,21] induced by γ-irradiation DNA, indicating that $H_2O^{\bullet +}$ or diffusible hydroxyl radicals, if produced by α-particles in the hydrated layer, do not contribute significantly to the induction of ssb, as discussed[20] previously. In contrast, the yield of DNA *prompt dsb* induced by α-particle irradiation increases with increasing level of DNA hydration at values of $\Gamma < 15$, compared with the independence on Γ for the corresponding yields induced by γ-irradiation. At the maximum level of $\Gamma$of 34.5, the yield of *prompt dsb* ($\sim 1.6 \pm 0.1 \times 10^{-11}$ ssb/Gy/Da) induced by α-particles is at least twice as large as that for γ-irradiation but similar to the overall yield of dsb[20] detected after an enzyme treatment for γ-irradiated plasmid. The ratio of ssb/dsb of 3−6 for α-particles is less than that obtained for γ-irradiation[20] (ssb/dsb ~ 10), probably reflecting the increased yield of dsb per ssb for α-radiation. The ratio of ssb/dsb of ~8 for α-particle irradiation[9] determined in aqueous solution containing high concentrations of radical scavenger (cell mimetic conditions) is greater than that determined in this study at a Γ of 34.5. Since direct energy deposition in the hydration layer of DNA produces an electron and $H_2O^{\bullet +}$, which do not produce additional ssb, the reasons for this increase in dsb are unclear at present. One possibility is that an increase in cluster complexity with hydration due to the formation of additional base lesions (see later) leads to an increase in the local destabilization of the hydrogen bonding so that the interbase pair separation between two bistranded ssb to give a dsb increases. Further, some of the increase in the yield of dsb at $\Gamma < 15$ may reflect conformational changes of DNA from the A to the B form. Independent of this increase with α-particles, these results clearly indicate that the yields of dsb at all values of Γ strongly depend on the ionization density of the radiation.

The absence of heat-labile sites which would have been seen as additional ssb on heating the irradiated DNA at 37 °C is consistent with our previous data[20] for γ-irradiation of hydrated plasmid DNA. Since direct ionization of the DNA−water complex does not result in heat-labile strand breakage, it is inferred that $OH^{\bullet}$ are the probable precursors to the induction of heat-labile sites in DNA irradiated in aqueous solution under cell mimetic conditions.[15]

Our previous study with γ-radiation of hydrated DNA[20] showed that the yield of ess increases with increasing level of hydration, in contrast to the lack of detectable amounts of ess induced by α-particles. These observations infer that either (i) base lesions are not induced in DNA by α-particles or (ii) the base lesions induced by α-particle irradiation are not converted into ssb by Nth and Fpg. The former case seems unlikely since base lesions are expected to be induced in hydrated DNA by α-particles. Indeed, DNA base modifications are induced in cells irradiated with high LET radiation.[43] They found that the types and relative yields of the different base lesions are similar for both high LET radiation and γ-radiation, but the overall yields of base lesions are ~50% lower when induced by high LET radiation. Further, base lesions, seen as ess, are induced by α-particle irradiation of DNA in aqueous solution in the presence of high concentrations of $OH^{\bullet}$ scavengers to provide cell mimic conditions with respect to the mean diffusion distance of ·OH.[9,28] It is therefore proposed that base lesions are mainly induced within clustered DNA damage by α-particle irradiation. If the majority of base lesions induced in hydrated DNA (direct effects) by α-particles are formed within clustered damage, it is predicted that the formation of ess would be severely retarded since excision of a base lesion within a clustered DNA damage by glycoylases is known to be inhibited.[44−48] Additionally, base lesions may also be formed in the vicinity of a prompt ssb or dsb so that ess from enzymatic treatment would not be seen. Theoretical studies using molecular dynamic approaches also predict conformational changes in DNA around the base lesion as well as disruption of hydrogen bonding. For instance, insertion of 7,8-dihydro-8-oxo-2′-deoxyguanine into a 15-mer oligonucleotide causes disruption of the hydrogen bonding network between base pairs near to the lesion and the base on the strand complementary to the lesion.[49] It was inferred that the structural and chemical changes of DNA around the damage site would be essential to guide repair proteins into the damage site. If there are additional base lesions induced within a few base pairs, these lesions may also coincide with the region recognized by the enzyme, so that additionally structural changes may result.

We showed[20] that the yield of clustered DNA damage induced by γ-irradiation and detected as additional dsb following enzyme treatment is at least twice that for prompt dsb. From the lack of observation of additional dsb following enzyme treatment in hydrated DNA irradiated with α-particles, it is proposed that not only are base lesions formed in clustered damage but also that the complexity of the clustered damage induced by α-particles is greater than that produced by γ-radiation. This increased complexity of clustered damage is consistent with differences in the ionization densities of these radiations and inhibition of excision of base lesions within a clustered DNA damage[44−48] to produce additional dsb for α-particle irradiation. Thus it is proposed that two types of clustered DNA damage are induced by direct effects in hydrated DNA using Nth and Fpg as probes, namely enzymatically susceptible and "enzyme-

silent" clustered damages. The former are induced mainly by sparsely ionizing radiation and seen as additional dsb following enzymatic treatment, whereas the latter are predominantly induced by densely ionizing radiation.

A consequence of clustered DNA damage is that its processing may be compromised, the extent of which depends on its complexity.[44−48] For instance the proportion of radiation-induced dsb rejoined in mammalian cells decreases on increasing the ionization density of the radiation.[3,50] These differences are thought to reflect the increased complexity of dsb (additional lesions in vicinity of dsb termini) on increasing ionization density of the radiation. Thus, it is important to consider how the spatial distribution of the lesions in DNA influences the biological consequences of the damage.

In summary, the yield of dsb induced by direct effects of α-radiation on hydrated plasmid DNA is twice that for γ-irradiation, indicating that dense ionization or excitation events along the α-particle tracks are more effective at inducing clustered types of strand breaks. Irrespective of the hydration level, α-particle irradiation of DNA induces more complex types of clustered DNA damage so that the majority of base lesions, which are refractory to excision by Fpg or Nth for α-radiation but not γ-radiation, are present in clustered DNA damage.

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