

# Induction of Single- and Double-Strand Breaks in Plasmid DNA by Monoenergetic Alpha-Particles with Energies below the Bragg-Maximum

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The yield of single-strand breaks (ssb) and double-strand breaks (dsb) produced by alpha-particles at the end of their track in DNA-films was determined experimentally. Helium nuclei were accelerated to 600 keV in the 400 kV ion accelerator and scattered at a carbon target. The elastically scattered alpha-particles with energies of 344 keV and 485 keV were used to irradiate supercircular plasmid DNA *in vacuo*.

For the dosimetry of the alpha-particles a surface barrier detector was used and the energy distribution of the alpha-particles determined. The energy loss of the particles in the DNA-layer was calculated. DNA samples were separated into the three conformational isomers using agarose gel electrophoresis. After fluorochromation the number of ssb and dsb per plasmid DNA molecule was established from the band intensities assuming the validity of Poisson statistics.

Linear dose effect correlations were found for ssb and dsb per plasmid molecule. In the case of 344 keV-alpha-particles the yield of dsb was  $(8.6 \pm 0.9) \times 10^{-11}$  breaks/Gy $\times$ dalton. The ratio of ssb/dsb was  $0.5 \pm 0.2$ . This is at least a factor of six larger than the ratio found in experiments with higher energy alpha-particles and from model calculations. Similar experiments with protons yielded a relative biological effectiveness (rbe) value of 2.8 for the induction of double-strand breaks by track end alpha-particles.

## Introduction

The interaction of ionising radiation with high linear energy transfer (LET) with DNA molecules leads to localised regions of damage at high density in the double-strand. According to detailed track structure analysis besides base damage, DNA protein crosslinks and intra-molecular DNA-crosslinks complex combinations of strand breaks, the so-called clustered-damage occurs (Goodhead, 1994). The importance of these lesions is that the cell can barely repair them, or not at all. This has been corroborated by irradiation studies with cells exposed to a lethal dose. After exposure to low-energy alpha-particles only 20 double-strand breaks per lethal event were

found in the cell nucleus whereas after hard X-rays almost 100 double-strand breaks have been detected (Fox and Prise, 1992).

A model developed by Sachs and Brenner (1993) indicates that misrepair of a special type of severe double-strand breaks can lead to exchange-type chromosomal aberrations such as dicentric chromosomes, translocations and inversions. With increasing LET of the used radiation the yield of the aberrations increases rapidly. Inversions may be responsible for cell transformation. This model is in agreement with numerous experimental studies on the biological effectiveness of alpha-particles in mammalian cells. It was found for example that in Chinese hamster ovary cells (CHO) after doses below 1 mGy sister chromatid exchanges take place (Nagasawa and Little, 1992) and that after irradiation of blood stem cells in mice the progeny of these cells carry multiple non-clonal chromosomal aberrations (Kadhim *et al.*,

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1992). In radiation studies involving various cell lines Raju *et al.* (1993) noticed that the efficiency of alpha-particles to inactivate cells decreases with decreasing particle energy. Specifically alpha-particle track ends in the descending part of the Bragg-curve are only half as lethal as particles with higher energies of the same LET on the ascending part of the Bragg-curve (Raju *et al.*, 1993). This condition applies, for example, to alpha-particles emitted from incorporated and inhaled radioisotopes, such as plutonium, radon and decay products, which are stopped in tissue by cells near the range of the particles, depositing their remaining energy of up to 700 keV in a single cell nucleus. In this connexion it should be noted that the dose due to radon in homes amounts to almost 30% of the total radiation burden of the average German citizen. According to a new compilation the radon concentration in the indoor air amounts to more than 250 Bq/m<sup>3</sup> in 2% of the homes (Bundesumweltministerium, 1993).

This study examines the correlation of radiation effects on the cellular level with those on the molecular level. In particular, we investigated whether the energy dependent and thus also LET dependent lethal and mutagenic effectiveness of alpha-particles can be correlated with the number and ratio of primarily direct induced single- and double-strand breaks. Since densely ionising radiation produces mostly complex combinations of ssb and dsb in the DNA the ratio of ssb/dsb will be taken as a measure of the severity of the damage. The LET dependence of dsb induction has been determined by using proton irradiation for comparison. The relative biological effectiveness (rbe) of alpha-particle track ends for dsb induction has been compared with the rbe of high energy alpha-particles as reported in the studies mentioned above.

## Materials and Methods

To exclude repair and indirect radiation effects, isolated plasmid DNA films were irradiated in vacuum and separately analysed for ssb and dsb. Alpha-particles and protons were generated by a 400 kV ion accelerator. Tuning of the required particle flux was achieved by elastically scattering the particles at a carbon target. A surface barrier detector was used for dosimetry.

### Plasmid preparation

DNA of a modified pUC-Vektor pBR 322 (Sutcliffe, 1979) with a total length of 7180 base pairs was selected for the irradiation experiments. This DNA is double-stranded, covalently closed and contains the gene for ampicillin resistance. It exists in the host bacterium almost exclusively in the supercircular form. The vector was propagated in an *E. coli* strain (HB101), known to have a high transformation rate thus allowing plasmid isolations with high yield.

Competent HB101 *E. coli* cells were transformed with plasmid-DNA and plated on ampicillin-containing LB-agarplates. For plasmid propagation, a transformed single colony of HB101 was incubated at 37 °C for 7 to 8 hours in 15 ml of LB-medium containing ampicillin (Sambrock *et al.*, 1989).

Plasmid-DNA was isolated by an optimized and rapid alkaline lysis method using Jet-Star-columns of GENOMED, Bad Oeynhausen. The bacterial cells were lysed under alkaline conditions and the plasmid DNA separated from cell debris and genomic DNA by the anion exchange columns (see instructions for the isolation procedure, Jet Star Midi Plasmid Purification System, Genomed, 1994). This procedure yielded about 150 µg plasmid-DNA per column with a 90% supercircular fraction. The DNA was dissolved in 10 mM Tris-EDTA-buffer at a final concentration of 0.5 µg/µl.

### Sample preparation

Sample holders with four wells each were cut out from round bottom microtiter plates (NUNC, Wiesbaden). Wells were loaded with 5 µl TE-buffer containing DNA and were stored for two hours in an exsiccator. Trials with stained DNA in TE-buffer and with stained buffer without DNA revealed that under those conditions the DNA is homogeneously distributed on the bottom of the well after drying.

The degree of DNA degradation after drying and redissolution was examined in a series of experiments using different buffers. Degradation was found to be negligible with Tris-EDTA-buffer. Samples were kept in an exsiccator under vacuum until use for irradiation.

### Irradiation

Plasmid DNA was irradiated using the 400 keV ion-accelerator of the Institute for Nuclear Physics of the University of Münster.  $\text{He}^{++}$ -ions were accelerated to energies up to 600 keV and protons up to 150 keV and, for adjustment of particle flux, were elastically scattered at a carbon target. The experimental setup of the irradiation chamber is shown in Fig. 1. Two collimators (B1 and B2) made of tantalum were used to collimate the primary beam to a diameter of 6 mm ensuring that

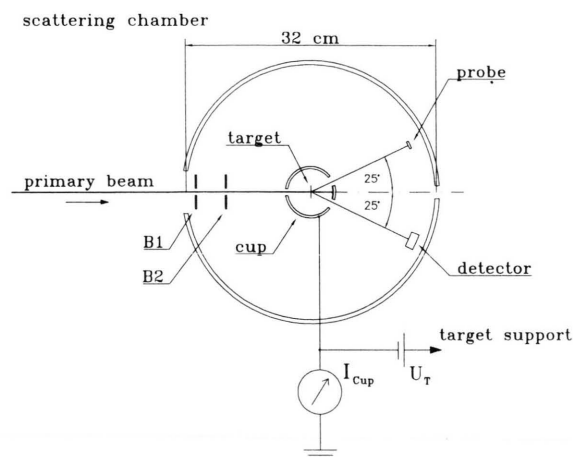


Fig. 1. Schematic drawing of the scattering chamber. The inner diameter of the chamber is 32 cm.  $I_{\text{cup}}$  = current measured in the Faraday cup;  $U_T$  = bias voltage of the target; B1, B2 = beam collimators.

the beam passes through the entrance hole of the Faraday cup and that it is focussed on the target center. Thus no primary particles could hit the cup from the outside. The Faraday-cup surrounding the target and the target support were mounted on to the top of the scattering chamber, electrically isolated. Due to this construction, a positive bias could be applied between target support and the Faraday cup in order to suppress secondary electron emission. The particle beam hitting the target could thus be measured. The two exit holes of the cup permitted exposure of the sample and detection of the particle beam under scattering angles of  $25^\circ$  symmetrical to the beam axis.

A surface barrier detector of type ORTEC BA-017-025-100 from E. G. & G. ORTEC, Oak Ridge, USA, with a resolution for alpha-particles of better than 17 keV served as particle counter and spectrometer.

Before each irradiation experiment The chamber was ventilated. After insertion of the sample holder the chamber was evacuated to a final pressure of  $5 \times 10^{-4}$  Pa. Each sample holder contained of two DNA-samples one of which was exposed. The other sample, covered by an aluminum foil, served as unirradiated reference probe. During the irradiation experiments with a maximal duration of 35 min the energy spectrum of the scattered particles was recorded to obtain both the particle energy and total particle flux. After exposure the samples were withdrawn from the flushed chamber and immediately analysed.

### Dosimetry

For the dosimetry a precise knowledge of the target thickness was necessary. The information provided by the manufacturer had an uncertainty of 50% and was, thus, insufficient. Therefore, proton scattering experiments were performed. Simultaneous measurements of incoming and scattered beam intensities yielded a target thickness of  $(18.6 \pm 2.4) \mu\text{g}/\text{cm}^2$ .

The energy  $E_T$  of the particles after scattering at the carbon target can be calculated according to

$$E_T = E_0 - dE_1 - dE_{\text{back}} - dE_2.$$

$E_0$  is the kinetic energy of the accelerated particles,  $dE_1$  and  $dE_2$  are the energy loss of the particles before and after the elastic scattering, respectively and  $dE_{\text{back}}$  the energy loss due to momentum transfer to the recoil nucleus. It was assumed that the collision with the target atom leading to the scattering of the particle takes place on the average half-way through the target. The energy calibration for alpha-particles was performed with three primary energies 200, 400 and 600 keV and at a scattering angle of  $25^\circ$ . The resulting analog-digital-counter (ADC) spectrum is shown in Fig. 2. A linear energy calibration is obtained for the range of incident energies as shown.

Since the DNA-samples were obtained from dried Tris-EDTA-buffer the sample wells contained homogeneous DNA-salt mixtures. It was, thus, possible to calculate the surface density in  $\mu\text{g}/\text{cm}^2$ . The calculation of energy loss of an alpha particle in this film and in plasmid DNA was obtained by considering the total layer to consist of

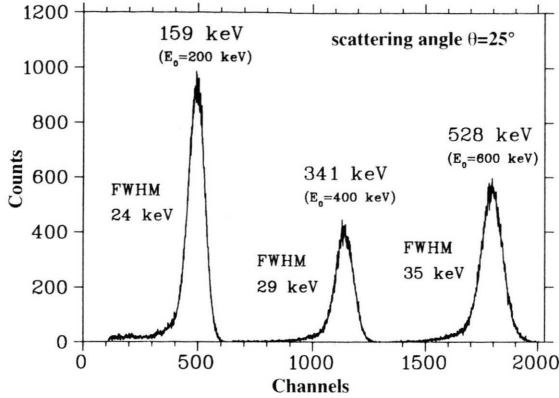


Fig. 2. The analog-digital counter-spectrum of alpha-particles with 3 different acceleration energies  $E_0$ . At the maxima the energies  $E_T$  after scattering at the carbon target are given and the fullwidth at half maximum (FWHM) as well.

$2n$  single layers with  $n$  thin DNA-layers interspersed with  $n$  salt layers.  $n$  was chosen large enough, so that the effective energy loss in the resulting single layer thickness could be taken to be constant. Thus, the energy deposition  $\Delta E_T$  of an alpha particle could be expressed as

$$\Delta E_T = \sum_i \frac{dE_i}{\rho dx} \times \rho dx$$

with the differential energy loss of the particle  $dE_i/\rho dx$  in the  $i$ -th DNA-layer and the DNA-layer thickness  $\rho dx$ . The differential energy losses were determined for each element separately by means of the computer code ASTAR. This program calculates the differential energy loss of alpha-particles in various elements normalized to material density, by interpolating experimental values of energy loss. The error of this method is estimated to be about 7% for the energy range from 300 to 600 keV. For calculating the total energy loss the single energy loss values were added according to the Bragg rule. Figure 3 shows the normalized differential energy loss of alpha-particles in DNA. The LET-values for the particle energies used in these experiments are shown as well. The absorbed dose ( $D$ ) in Gray can thus be expressed as

$$D = 1.6 \times 10^{-10} \frac{\Delta E_T}{\Delta m} n_T$$

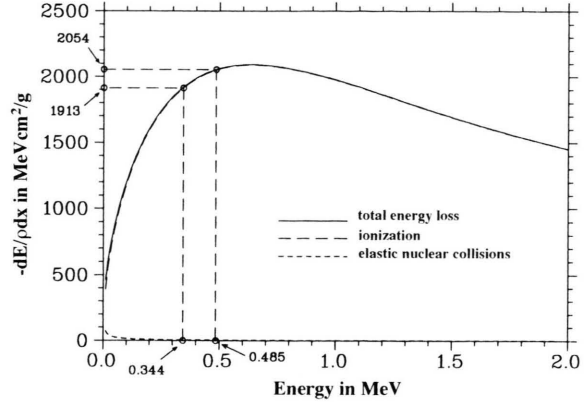


Fig. 3. Normalized differential energy loss ( $dE_T/\rho dx$ ) of alpha-particles in DNA is given as function of particle energy. Energy loss due to ionization, elastic nuclear collision and the sum of both effects are shown.

where  $\Delta m$  denotes the DNA mass and  $n_T$  the total number of particles.

#### Analysis of strand breaks

The analysis of plasmid DNA for ssb and dsb was performed using agarose-gel electrophoresis. After an irradiation series the DNA samples were redissolved in 8  $\mu$ l of  $H_2O$ , diluted 1:40 and 10  $\mu$ l of the final solution (about 80–100 ng DNA) was pipetted into each sample well of an one% agarose gel. Using a buffer solution containing 50 mM Tris/HCl (Tris[hydroxymethyl]-aminomethane hydrochloride), 20 mM sodium acetate and 2 mM EDTA, electrophoresis for three hours at a field strength of 4 V/cm was sufficient to separate the three conformational isomeric DNA forms (linear (lin), opencircular (oc) and supercircular (sc)). Gels were stained in  $H_2O$  containing 0.5  $\mu$ g/ml ethidium bromide for 1 hour to visualize the well separated DNA bands. After rinsing with distilled water the gels were photographed with a polaroid PM-4 camera using a 102 x 125 mm negative film (Agfa pan 400 professional). With an exposure time of 140 s the fluorescence intensities of the DNA bands were in the linear range of a simultaneously photographed step wedge. This allowed quantification of the DNA bands with a laser densitometer (Ultrascan XL, LKB/Pharmacia). The area of the DNA peaks of the linear, opencircular and supercircular forms were determined by approximation with a Gauss function. The peak area

was taken as a measure of the quantity of DNA in the band.

For calculating the number of induced ssb and dsb could then be calculated assuming applicability of Poisson-statistic for strand break induction. The sum of ssb and dsb assuming single hit kinetics is given by:

$$n_{ssb} + n_{dsb} = \ln(N_0/),$$

where  $N_0$  is the number of exposed sc DNA molecules and  $N$  the number of remaining sc DNA molecules after a given dose. The average number of dsb per plasmid molecule can be calculated from the first term of the Poisson distribution:

$$P(1) = n_{dsb} \times \exp(-n_{dsb}) = F_{lin} / (F_{sc} + F_{oc})_{after\ irradi.}$$

Here  $F_{lin}$ ,  $F_{sc}$  and  $F_{oc}$  are the fractions of the linear, supercircular and opencircular plasmid-DNA molecules, respectively. The three fractions  $F_{sc}$ ,  $F_{oc}$  und  $F_{lin}$  could then be obtained from the relative decrease of the sc-form and the increase of the oc- and lin- forms of the irradiated samples compared to the unirradiated control (Menke and Köhnlein, 1992).

## Results

### Irradiation spectra

Table I presents an overview of the plasmid irradiation series with alpha-particles and protons.  $E_T$  is the energy of the particles after scattering at the carbon target and  $\Delta E_T$  the energy loss of the particle in the DNA sample. The energy spectra of

Table I: Overview of the plasmid–DNA irradiation experiments. The sample thickness was  $(34.1 \pm 3.1) \mu\text{g}/\text{cm}^2$  for the 344 keV–alpha particle irradiation and  $(26.1 \pm 2.6) \mu\text{g}/\text{cm}^2$  for the other irradiations, respectively.

Acceleration energy of the particles $E_0^{\text{ion}}$	Particle energy $E_T$ after scattering [keV]	Energy loss in the DNA $\Delta E_T$ [keV]
600 keV $\text{He}^{++}$	$485 \pm 20$	$50.2 \pm 0.6$
400 keV $\text{He}^{++}$	$344 \pm 14$	$56.4 \pm 1.3$
150 keV protons	$135 \pm 10$	$19.1 \pm 0.4$
100 keV protons	$87 \pm 10$	$20.6 \pm 0.2$

the two irradiation series with alpha-particles are shown in Fig. 4. The energy resolution in both cases was  $\Delta E/E_0^{\text{ion}} = (8.1 \pm 0.3) \times 10^{-2}$ , where  $\Delta E$  is the full width at half maximum of the peak. In addition to the peak of the alpha-particles a second peak is clearly visible at about a quarter of the energy of the alpha particles. This peak is caused by protons which are generated in the carbon target by scattering of  $\text{H}_2^+$ -molecule ions.

Since  $\text{H}_2^+$ -molecule ions, which are generated together with the  $\text{He}^{++}$ -ions in the “Penning” ion source of the accelerator have the same beam rigidity

$$R = \sqrt{\frac{A \times E_{kin}}{q}}$$

where  $A$  = nuclear mass number;  $E_{kin}$  = kinetic energy and  $q$  = charge of the accelerated particle, they could not be separated from the  $\text{He}^{++}$ -ion beam by means of an analysing magnet.

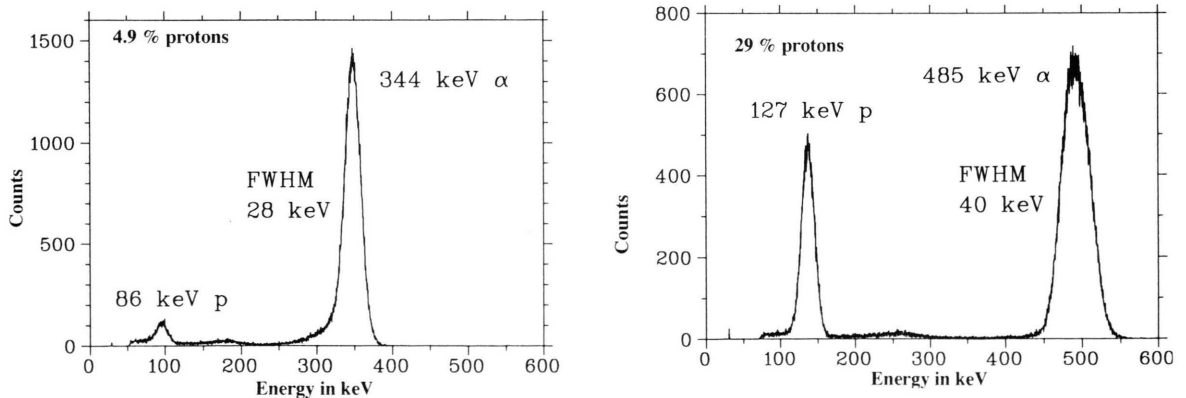


Fig. 4. Energy spectra of the 344 keV (a) and 485 keV–alpha–particles (b). The number of scattered particles is plotted versus their energy. The mean energies for the peaks are also given. The peaks at 86 keV (a) and at 127 keV (b), respectively, are due to contaminating protons (p) see also Results.



Ratio of DNA strand breaks

For all irradiation experiments the number of ssb and dsb per plasmid DNA molecule per absorbed dose is given in Fig. 5. Although the individual experimental values scatter it was assumed that ssb and dsb are linearly correlated with dose. With 344 keV-alpha particle irradiation, notice that the number of dsb at a given dose is almost twice the number of ssb, where as with 485 keV alpha radiation there are more ssb than dsb at the same dose, even though the LET of the alpha-particles of both energies is the same within the experimental accuracy (see also Table II).

To understand these results it was necessary to conduct irradiation experiments with “pure” proton radiation. The acceleration energy  $E_0^{\text{ion}}$  was selected such that proton energy was half the energy of  $\text{H}_2^+$ -molecules, which are coaccelerated with alpha-particles. The results of these experiments are also shown in Fig. 5. With protons the

Table II. For the used particles the yield of single-strand breaks and double-strand breaks per dose unit and dalton ( $m(\text{ssb})$  and  $m(\text{dsb})$ , respectively) are given together with the ratio  $\text{ssb}/\text{dsb}$  and mean LET in  $\text{MeV}\times\text{cm}^2/\text{g}$ .  $m(\text{ssb})$  and  $m(\text{dsb})$  are obtained from the corresponding slopes in Fig. 5(a–d). The molecular weight of the plasmid–DNA was:  $\text{MG}_{\text{Plasmid}} = 4.67\times10^6$  dalton.

Strandbreaks, ratio, LET	344 keV alpha-particles	485 keV alpha-particles
$m(\text{ssb})$	$(4.3 \pm 1.1)\times10^{-11}$	$(6.0 \pm 2.1)\times10^{-11}$
$m(\text{dsb})$	$(8.6 \pm 0.9)\times10^{-11}$	$(7.1 \pm 0.9)\times10^{-11}$
$\text{ssb}/\text{dsb}$	$0.5 \pm 0.2$	$0.8 \pm 0.4$
LET	$1659 \pm 189$	$1923 \pm 214$

Strandbreaks, ratio, LET	87 keV protons	135 keV protons
$m(\text{ssb})$	$(9.9 \pm 0.4)\times10^{-11}$	$(1.1 \pm 0.1)\times10^{-10}$
$m(\text{dsb})$	$(3.0 \pm 0.2)\times10^{-11}$	$(2.6 \pm 0.4)\times10^{-11}$
$\text{ssb}/\text{dsb}$	$3.3 \pm 0.4$	$4.2 \pm 1.0$
LET	$789 \pm 86$	$732 \pm 88$

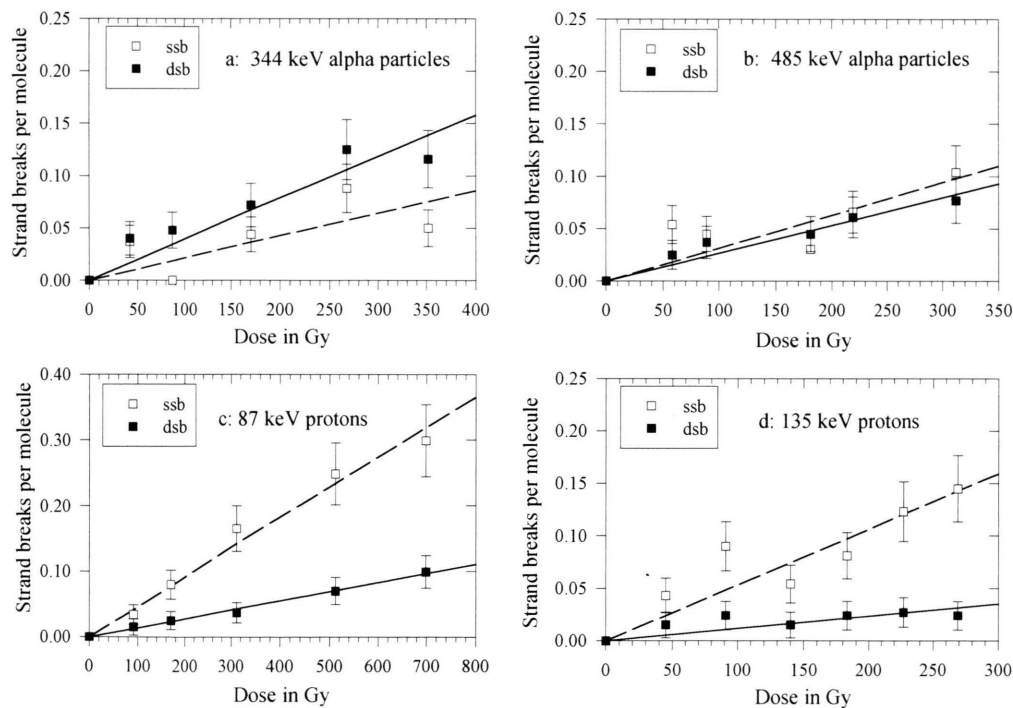


Fig. 5. Dose effect relationships for single-strand breaks (ssb) and double strand breaks (dsb) are shown for the various irradiation experiments (--- dsb, — ssb). From the slopes of the curves the yield for ssb and dsb can be calculated (see Table II). The parts a to d show the dose response curves for the production of single and double strand breaks for the indicated irradiations with alpha-particles and protons.

induction of ssb is larger by a factor of 3 to 4 than the induction of dsb. This is consistent with the results of alpha irradiation in which the induction of ssb is predominant if the particle beam is contaminated with a high proton content. We, therefore, concluded that the high yield of ssb with 485 keV alpha radiation can be attributed to the relatively high proton fraction in the beam.

It was, therefore, necessary to correct our calculation of ssb and dsb yields per molecule and dose as sum of yields for pure alpha and proton radiation weighted for their respective contributions. The corrected final results for alpha and proton irradiation are compiled in Table II. Alpha-particles with energies up to and including the Bragg maximum produce in DNA directly up to twice as many dsb than ssb. With protons (87 keV) with LET a factor of 2.4 smaller at the Bragg maximum than 485 keV alpha-particles we observed 1 dsb per 3 induced ssb's. Thus, we obtain a relative biological effectiveness (rbe) of 2.8 for alpha particles at the Bragg maximum for producing double-strand breaks, as compared to protons.

### Energy transfer

The mean energy transferred per strand break could be derived from the dose effect relations for ssb and dsb induction and from the known number of irradiated plasmid DNA molecules. In doing so we assumed that each interaction of an alpha particle or proton with a supercircular plasmid molecule leads to a strand break.

For estimating the energy deposition per strand break the value of the  $D_{90}$  was used. At that dose 90% of the supercircular Plasmid molecules have not yet suffered a strandbreak. The mean energy depositions per strand break for the various irradiation experiments are given in Table III. The relatively large figures for the  $D_{90}$  values result from the small molecular weight ( $MG_{\text{Plasmid}} = 4.67 \times 10^6$  dalton) of the plasmid DNA used.

### Discussion

The presented results show that for irradiation experiments with low energy alpha-particles and protons it is advantageous to use an ion accelerator in connection with a scattering chamber. With the described arrangement monoenergetic alpha-particles with energies below 500 keV and a sharp

Table III. The mean energy  $E_s$  transferred per DNA strandbreak as calculated from the  $D_{90}$  the dose where 90% of the supercircular DNA molecules survive without strandbreak.

Radiation	$D_{90}$ [Gy]	$E_s$ [eV]
344 keV alpha-particles	$167 \pm 26$	$80 \pm 15$
485 keV alpha-particles	$164 \pm 37$	$79 \pm 20$
87 keV protons	$161 \pm 6$	$77 \pm 10$
135 keV protons	$154 \pm 16$	$74 \pm 10$

energy distribution of  $\Delta E/E_0^{\text{Ion}} = (8.1 \pm 0.3) \times 10^{-2}$  were obtained. In contrast the energy distribution using radioactive alpha emitters is wider by a factor of 25–30 (Eisen *et al.*, 1991). Irradiating dried DNA samples in the vacuum proved to be of advantage by suppressing the indirect radiation effect due to water radicals. On the other hand the contamination of the primary  $\text{He}^{++}$ -beam with  $\text{H}_2^+$ -molecules was a disadvantage in our experiments. This is, however, a specific problem of the ion accelerator used, since it lacks an appropriate beam filter. Also, for dose calculations direct measurements of energy loss of alpha-particles in a DNA-film would be preferable. Saltfree DNA samples would also be of advantage.

The results of our irradiation experiments show, that alpha-particles with energies from 344 to 485 keV induce strand breaks in plasmid DNA by direct interaction, 60% of which are dsb and 40% ssb. The smallest ratio ssb/dsb measured was  $0.5 \pm 0.2$  at a dsb-yield of  $(8.6 \pm 1.0) \times 10^{-11}$  breaks / Gy · dalton with 344 keV-alpha radiation. Corresponding values of  $3.3 \pm 0.4$  (87 keV-protons) and  $4.2 \pm 1.0$  (135 keV-protons) were found, respectively, with proton radiation (Table II). Per strand break event (ssb and dsb) an energy transfer to the plasmid molecule of  $80 \pm 14$  eV from an alpha particle and  $76 \pm 10$  eV from a proton was obtained.

Comparing these results with previously published work in which phage DNA was irradiated with alpha-particles of high energies, we conclude that with increasing particle energy and simultaneously decreasing LET the yield of dsb decreases. Irradiating films of T7 phage DNA with 5 MeV alpha-particles with an LET value of about 900 MeV · cm<sup>2</sup>/g yields  $1.1 \times 10^{-11}$  dsb/Gy · dalton (Neary *et al.*, 1972), whereas a value of only  $0.21 \times 10^{-11}$  dsb/Gy · dalton was observed by Chris-

tensen *et al.* (1972) after exposing the replicating form of  $\Phi$ X-137 phage DNA in nutrient broth to 30 MeV alpha-particles with an LET of  $343 \text{ MeV} \times \text{cm}^2/\text{g}$ . These values are smaller by a factor of 8 and 40, respectively, than the values reported here. Monte-Carlo simulations of ion tracks in a DNA segment of 54 basepairs revealed for 1.2 MeV alpha-particles (LET  $\sim 2000 \text{ MeV} \cdot \text{cm}^2/\text{g}$ ) a dsb rate of  $\sim 2.7 \times 10^{-11} \text{ dsb/Gy} \cdot \text{dalton}$  (Charlton *et al.*, 1989). This is smaller by a factor of 3 than the value observed here. In all of the above cited papers the ratio ssb/dsb is larger than 1 and at least larger by a factor of 6 compared to the ratio found with 344 keV alpha particle radiation.

It was proposed (Goodhead, 1994; Ward, 1985) that a small ssb/dsb ratio indicates the presence of clustered damage. Thus the steady decrease of the ratio ssb/dsb and the increase of the dsb yield with decreasing energy of the alpha-particles is an indication for increasing complexity of clustered damage beyond the Bragg maximum to the end of the track ( $E_T < 500 \text{ keV}$ ).

A broad spectrum of complex lesions is generated. Thus the probability for inducing strand breaks, that may lead to DNA exchange reactions is increasing (Sachs and Brenner, 1993), while

simultaneously the amount of lethal damage is decreasing.

Such an mechanism could explain the observation that alpha-particles coming to rest in the cell nucleus are only half as lethal than those penetrating the cell nucleus (Raju *et al.*, 1993).

As already mentioned there are numerous indications in the literature, that different types of particle radiation induce lesions in the cellular DNA detectable as dsb which have different probabilities of biological consequences for the cell. For example irradiation experiments with V79 cells using alpha-particles and protons of the same LET lead to similar yields of dsb. For protons, however, with respect to cell inactivation the linear term of the dose effect curve is significantly larger. The mutation induction was also found to be increased by a factor of two (Jenner *et al.* 1992; Bellini *et al.* 1992).

These correlations should be elucidated further with irradiation experiments using mammalian cells and alpha-particles with different energies, on either side of the Bragg maximum, but with identical LET with point mutations and chromosomal aberrations as endpoints. This would lead to a direct correlation between dsb and mutations.

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