Acta Oncologica Vol. 35, No. 7, pp. 797–801, 1996

STRAND BREAKS IN PLASMID DNA FOLLOWING POSITIONAL CHANGES OF AUGER-ELECTRON-EMITTING RADIONUCLIDES

S. JAMES ADELSTEIN and AMIN I. KASSIS

The purpose of our studies is to elucidate the kinetics of DNA strand breaks caused by low-energy Auger electron emitters in close proximity to DNA. Previously we have studied the DNA break yields in plasmids after the decay of indium-111 bound to DNA or free in solution. In this work, we compare the DNA break yields in supercoiled DNA of iodine-125 decaying close to DNA following DNA intercalation, minor-groove binding, or surface binding, and at a distance from DNA. Supercoiled DNA, stored at 4°C to accumulate radiation dose from the decay of ^{125}I, was then resolved by gel electrophoresis into supercoiled, nicked circular, and linear forms, representing undamaged DNA, single-strand breaks, and double-strand breaks respectively. DNA-intercalated or groove-bound ^{125}I is more effective than surface-bound radionuclide or ^{125}I free in solution. The hydroxyl radical scavenger DMSO protects against damage by ^{125}I free in solution but has minimal effect on damage by groove-bound ^{125}I.

There is general agreement that under certain circumstances Auger-electron-emitting radionuclides can be extremely radiotoxic and produce extensive DNA damage, including the shattering of both strands of duplex DNA and of poly- and oligonucleotides. The degree of damage appears to depend upon the location of the decaying atom and microdosimetric calculations predict such. For example, the cytoplasmic dye iodorhodamine, labeled with ^{125}I, produces in Chinese hamster cells a survival curve that resembles low-LET irradiation (1). When covalently bound to DNA, ^{125}I produces a high-LET curve under the same experimental circumstances (2), while intercalated ^{125}I produces a high-LET curve with RBE 50 to 60% that of the incorporated radionuclide (3). We have also found that ^{123}I incorporated into mammalian cell DNA produces about 0.7 double-strand breaks (dsb) per decay (4) in keeping with theory and the original observation that each decay of ^{125}I produces one dsb (5).

In molecular systems, Martin & Haseltine (6) have shown that covalently linked ^{125}I shatters both ipsilateral and contralateral strands of DNA in duplex oligonucleotides. Martin and his colleagues have further determined that the aminoacridine iodorivanol, a DNA intercalator, when labeled with ^{125}I, generates dsb in DNA (7). Linz & Stocklin (8) have also demonstrated that ^{125}I covalently bound to plasmid DNA is more efficient than ^{125}I free in solution at causing dsb, while we have shown that ^{111}In in its ionic form, bound to the outer side of DNA through the phosphate and nitrogenous base groups of the nucleotides (9), causes the loss of supercoiled pBR322 and the production of single-strand breaks (ssb) with 10 to 20 times the efficiency of the isotope kept free in solution by coordination with DTPA (10). Panyutin & Neumann (11) have recently reported that an ^{125}I-labeled, specific triplex-forming oligonucleotide, binding to the major groove, produces dsb with an efficiency of 0.8.

Four mechanisms for the production of dsb have been proposed (12): double-strand breaks from two nearby single-strand breaks (ssb) on opposite strands (the quadratic two-event mechanism); dsb from radical transfer

Received 16 October 1995.
Accepted 26 February 1996.
From Harvard Medical School, Department of Radiology, Boston, MA, USA.
Correspondence to: Dr. S. James Adelstein, Shields Warren Radiation Laboratory, 50 Binney Street, Boston, MA 02115, USA.
Paper presented at the Third International Symposium on Biophysical Aspects of Auger Processes, August 24–25, 1995, Lund, Sweden.

 ISSN 0284-186X

Table 1

Positional decay of Auger-electron-emitting radionuclides

Type	Example
Minor groove binding	^{125}I-Hoechst 33342
Intercalation	2-[^{125}I]iodoacridine
Outside binding	4-[^{125}I]iodoacridine
Free	[^{125}I]iodoantipyrine

between strands (Siddiqi--Bothe hypothesis, (13)); dsb from clusters of watery OH radicals from a single event (Ward's multiply damaged site hypothesis, (14)); and direct action on both strands by a single densely ionizing event. It has been postulated that scavenging molecules will protect against the first two but not the last two mechanisms.

In order to explore the dependence of DNA damage on the position of the decaying Auger-electron-emitting atom we have employed two well-characterized plasmids, pBR322 and pUC19. In their native form these plasmids consist of supercoiled DNA; a ssb converts them to a circular (nicked) form and a dsb to a linear form. The three forms are easily separated by electrophoresis on agarose gels. In our studies, a number of chemical entities have been used to explore radiolytic effects, i.e. damage to plasmid DNA. The radiochemicals include: iodinated Hoechst dye 33342 (H33342) for groove-bound ^{125}I; 2-iodoacridine for intercalated ^{125}I; 4-iodoacridine for non-specifically bound ^{125}I; iodoantipyrine for ^{125}I free in solution; (Table 1). In two of these systems (^{125}I-H33342 and [^{125}I]iodoantipyrine), the radioprotective effects of DMSO were also studied.

Material and Methods

Preparation of plasmids

The pBR322 plasmid was grown in E. coli (recA, thi$^-$) bacteria in L.B. medium containing 100 μg/ml ampicillin at 37°C and the pUC19 plasmid in E. coli (DH5 α) in T.B. medium containing ampicillin (150 μg/ml) and thiamine (10 μg/ml). The plasmids were amplified (pBR322 for 18 h in the presence of 5 μCi/ml ^{3}H-thymidine and pUC19 for 42 h in the presence of 36 μCi/ml ^{3}H-thymidine) and then purified on a commercially available resin column (Qiagen Incorporated, Studio City, CA).

Preparation of radiochemicals

2-Iodoacridine and 4-iodoacridine were synthesized as previously described (15). [^{125}I]iodoantipyrine was purchased from ICN Pharmaceuticals, Incorporated (Costa Mesa, CA). The method for iodinating Hoechst dye 33342 will be published in the near future.

Experimental protocols

The individual protocols are summarized in Table 2. pBR322 and pUC19 plasmids (100 μg) prepared as described above were placed in solution (final volume = 0.1 ml) containing the radiochemicals listed in Table 1, and the samples were stored at 4°C to accumulate radiation dose (1–60 days). The ^{125}I-labeled iodoacridines along with 0.2% acetone were dissolved in 10 mM sodium phosphate buffer, pH 4.9, containing 0.1 mM EDTA and mixed with pBR322 plasmids that were extensively dialyzed in the same buffer immediately prior to use. When ^{125}I-H33342 and [^{125}I]-iodoantipyrine were compared, pUC19 plasmids were mixed with each of these agents in phosphate-buffered saline, pH 7.4, and in some instances DMSO (0.2 M) was added to assess the effects of hydroxyl radical scavenging.

DNA strand-break assay

After the accumulation of the desired radiation dose from the decay of the radioisotope, the plasmid DNA samples were resolved by gel electrophoresis into supercoiled, nicked circular, and linear forms, representing undamaged DNA, ssb, and dsb respectively. Distinct bands of DNA formed when one microgram of DNA from each sample was applied on 0.8% agarose gel and the electrophoresis apparatus was run for 18 h (3 volts/cm) in the dark in TBE buffer (89 mM tris, 89 mM borate, 1 mM EDTA), pH 8.0, containing 0.5 μg/ml ethidium bromide. The agarose gels were washed in phosphate buffer, photographed (under UV), and the DNA-containing bands excised under UV light and dissolved in 0.5 ml of 1 M HCl by heating at 60°C for 15 min. In the radiolabeled acridine studies, the concentration of ^{3}H-DNA in the DNA bands, which is proportional to the amount of DNA present, was determined by assaying the radioactivity in a liquid scintillation counter. Wherever needed, the counts were corrected for ^{125}I spill over. In the ^{125}I-iodoantipyrine and ^{125}I-labeled H33342 studies, the DNA content within each band was estimated visually by two independent observers.

Table 2

Experimental protocols

pBR322			pUC19 (± DMSO)		
Intercalated 2-[^{125}I]iodoacridine	versus	Bound 4-[^{125}I]iodoacridine	Groove-bound ^{125}I-Hoechst 33342	versus	Free [^{125}I]iodoantipyrine

Results and Discussion

The experiments reported herein address the production of single- and double-strand breaks under various circumstances.

DNA-bound and intercalated ^{125}I

Acridines and proflavines, flat aromatic compounds, interact with DNA by sandwiching themselves between the stacked bases at right angles to the long axis of the helix (16). If a bulky iodine atom is attached to the leading edge of such molecules, as with 4-iodoacridine, the planar molecule cannot enter the stack; if the iodine is attached to the trailing edge, as with 2-iodoacridine, intercalation can proceed. 4-Iodoacridine when mixed with DNA shows a small hypochromic shift but no bathochromic one, signifying binding but not intercalation, while 2-iodoacridine demonstrates a bathochromic shift with an isosbestic point indicating that intercalation has taken place (15). Incubation of the two ^{125}I-labeled compounds with plasmid DNA in the presence of acetone, an hydroxyl radical scavenger, shows efficient dsb formation with the 2-iodo compound (0.67 dsb on average per disintegration) and much less dsb production with the 4-iodo compound (0.27 dsb on average per disintegration) (Table 3). The ssb/dsb ratio changes from 0.6 with 2-iodoacridine to approximately 16 with 4-iodoacridine.

Groove-bound and free ^{125}I

The *bis*-benzimidazole dyes, Hoechst 33258 and 33342, have been shown to bind to the minor groove of DNA in A-T rich regions (17). A radioiodinated analog of Hoechst 33342 has recently been synthesized and characterized. It has been shown to concentrate in the nuclei of mammalian cells (to be published). When the dyes are iodinated and mixed with DNA, the iodo group is located between the two strands of DNA in the minor groove (18). Iodination does not change the binding constant or the maximum number of molecules bound to DNA (Table 4). The DNA of plasmids exposed to ^{125}I-labeled Hoechst 33342 has been compared with that exposed to [^{125}I]iodoantipyrine, a diffusible, water-soluble compound. Inspection of the gels from plasmid--iodoantipyrine incubation mixtures reveals a rapid conversion of supercoiled to nicked (ssb) DNA with the progressive appearance of linear DNA (dsb) (Fig. 1). Inspection of the gels from plasmid--iodinated Hoechst 33342 incubation mixtures shows the simultaneous appearance of the nicked and linear forms, indicating the direct formation of dsb (Fig. 1). Semiquantitative visual analysis reiterates these findings and demonstrates that after an equal number of decays, about five times as many dsb are produced in DNA by the radioiodinated groove binder as by [^{125}I]iodoantipyrine (Fig. 2). Furthermore, addition of the hydroxyl radical scavenger DMSO leads to a substantial reduction in the rate of ssb production by iodoantipyrine and virtually no dsb production, while DMSO has little effect on either ssb or dsb production by the groove

Table 4

Characteristics of groove-binding Hoechst compounds

Ligand	K_a ($\times 10^7$ M^{-1})	Ligand/nucleotide
Hoechst 33258	2.64	0.0275
Hoechst 33342	2.84	0.0282
^{125}I-H 33342	2.57	0.0280

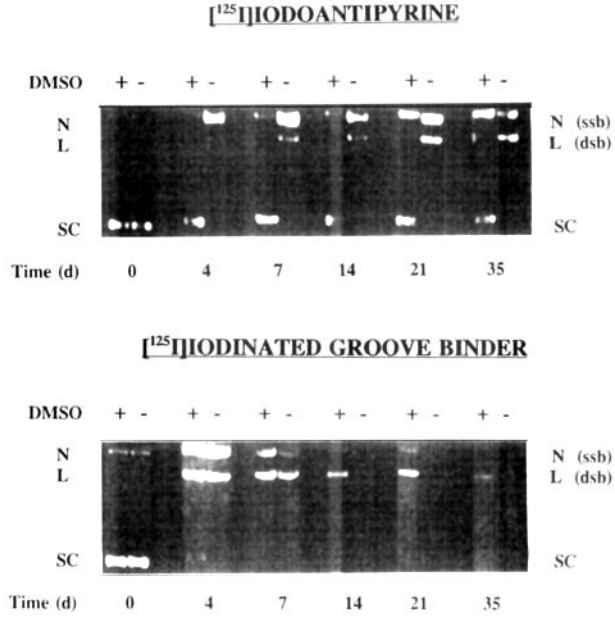

Fig. 1. Agarose gels of pUC19 supercoiled plasmid DNA exposed over time to ^{125}I-labeled Hoechst 33342 and [^{125}I]iodoantipyrine with and without DMSO; SC = supercoiled, N = nicked (ssb), L = linear (dsb). Note disappearance of total plasmid DNA from gel over time course of experiment.

Table 3

DNA strand breaks per disintegration of ^{125}I-labeled iodoacridines

DNA	2-[^{125}I]iodoacridine	4-[^{125}I]iodoacridine	2-[^{125}I]iodoacridine / 4-[^{125}I]iodoacridine
ssb	0.38	4.33	0.09
dsb	0.67	0.27	2.46

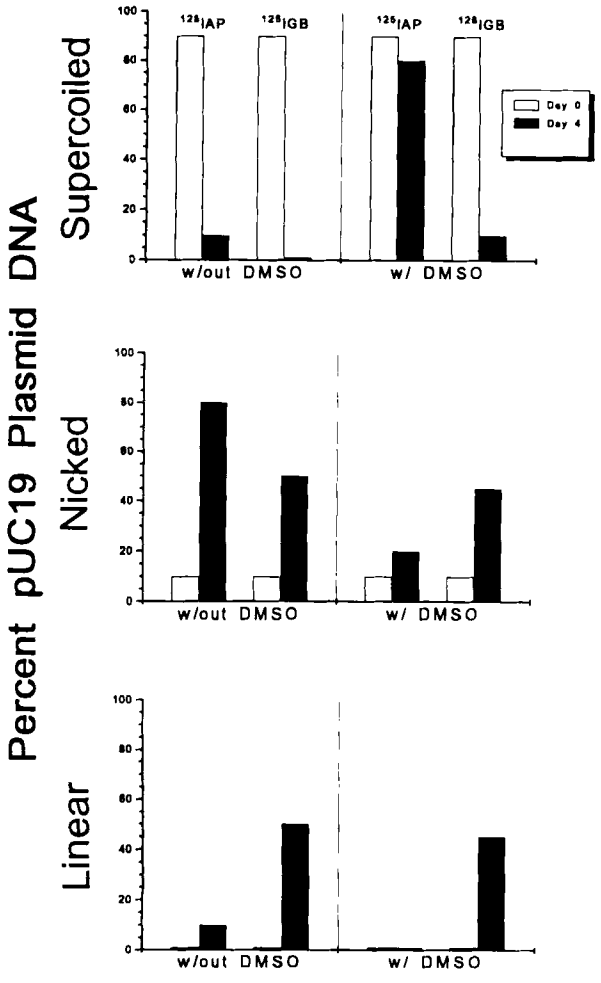

Fig. 2. Semiquantitative analysis of gels (days 0 and 4 ± DMSO) in Figure 1. Percentages take into account only three major species of DNA (supercoiled, nicked, and linear) and not other plasmid breakdown products.

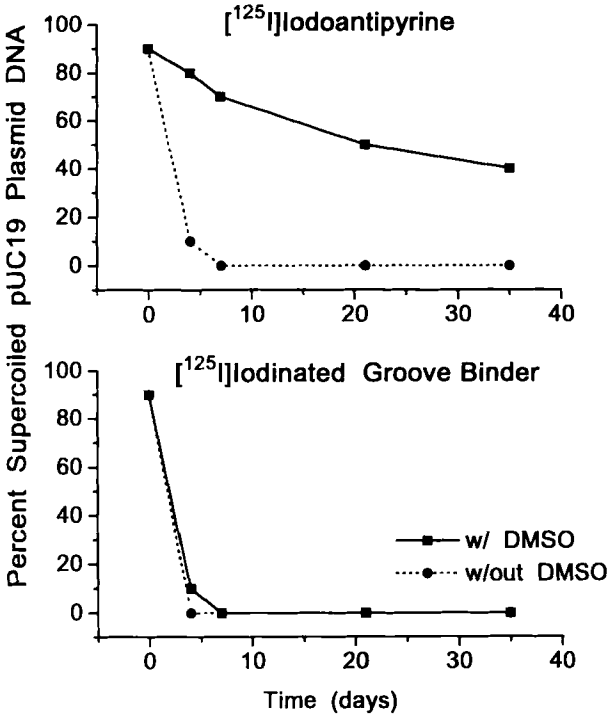

Fig. 3. Percentage of supercoiled DNA remaining over time after exposure of pUC19 plasmid DNA to ^{125}I-labeled Hoechst 33342 and [^{125}I]iodoantipyrine with and without DMSO (data generated from Fig. 1). Percentages take into account only three major species of DNA (supercoiled, nicked, and linear) and not other plasmid breakdown products.

binder (Figs. 2 and 3). This observation is consistent with dsb production that is caused by radical clusters or direct action by the ^{125}I affixed to the groove binder, i.e. in close proximity to DNA.

Conclusions

In plasmid DNA, damage caused by Auger electron emitters depends strongly on the position of the decaying radionuclide relative to the supercoiled structure. Our observations include the following:

- Ionic ^{111}In, bound on the surface of DNA through the phosphate and nitrogenous base groups of nucleotides, destroys the supercoiled form of pBR322 and produces ssb with 10 to 20 times the efficiency of the isotope not bound and kept free in solution by coordination with DTPA (10).
- Intercalated ^{125}I (2-[^{125}I]iodoacridine) is two to three times as effective as bound but unintercalated ^{125}I (4-[^{125}I]iodoacridine) in producing dsb (15).
- Groove-bound ^{125}I (radioiodinated Hoechst 33342) is five to seven times more efficient than free ^{125}I ([^{125}I]iodoantipyrine) in producing dsb.
- The hydroxyl radical scavenger DMSO protects against damage by free ^{125}I ([^{125}I]iodoantipyrine) but has little influence on damage produced by groove-bound ^{125}I (radioiodinated Hoechst 33342).

These observations are consistent with microdosimetric analyses and the production of dsb by a single-event mechanism related to the high radical density produced by the decaying Auger-electron-emitting atom when in close proximity to DNA as found in intercalation and minor-groove binding. We predict the same when ^{125}I is covalently bound.

ACKNOWLEDGEMENTS

We wish to thank our colleagues, past and present, for their contributions to these experiments: J. Baranowska-Kortylewicz, R. S. Harapanhalli, Z. P. Kortylewicz, G. M. Makrigiorgos, S. K. Sahu. This work was supported by NIH grant CA 15523.

REFERENCES

1. Kassis AI, Fayad F, Kinsey BM, Sastry KSR, Taube RA, Adelstein SJ. Radiotoxicity of ^{125}I in mammalian cells. Radiat Res 1987; 111: 305–18.

2. Kassis AI, Sastry KSR, Adelstein SJ. Kinetics of uptake, retention, and radiotoxicity of ^{125}IUdR in mammalian cells: implications of localized energy deposition by Auger processes. Radiat Res 1987; 109: 78–89.
3. Kassis AI, Fayad F, Kinsey BM, Sastry KSR, Adelstein SJ. Radiotoxicity of an ^{125}I-labeled DNA intercalator in mammalian cells. Radiat Res 1989; 118: 283–94.
4. Makrigiorgos GM, Berman RM, Baranowska-Kortylewicz J, et al. DNA damage produced in V79 cells by DNA-incorporated iodine-123: a comparison with iodine-125. Radiat Res 1992; 129: 309–14.
5. Krisch RE, Ley RD. Induction of lethality and DNA breakage by the decay of iodine-125 in bacteriophage T_4. Int J Radiat Biol 1974; 25: 21–30.
6. Martin RF, Haseltine WA. Range of radiochemical damage to DNA with decay of iodine-125. Science 1981; 213: 896–8.
7. Martin RF, Bradley TR, Hodgson GS. Cytotoxicity of an ^{125}I-labeled DNA-binding compound that induces double-, stranded DNA breaks. Cancer Res 1979; 39: 3244–7.
8. Linz U, Stocklin G. Chemical and biological consequences of the radioactive decay of iodine-125 in plasmid DNA. Radiat Res 1985; 101: 262–78.
9. Kalfas CA, Sideris EG, Martin PW. Perturbed γ–γ angular correlation studies of ^{111}In bound to double and single stranded DNA. Int J Appl Radiat Isot 1984; 35: 889–93.
10. Sahu SK, Kassis AI, Makrigiorgos GM, Baranowska-Kortylewicz J, Adelstein SJ. The effects of indium-111 decay on pBR322 DNA. Radiat Res 1995; 141: 193–8.
11. Panyutin IG, Neumann RD. Sequence-specific DNA double-strand breaks induced by triplex forming ^{125}I labeled oligonucleotides. Nucleic Acids Res 1994; 22: 4979–82.
12. Krisch RE, Flick MB, Trumbore CN. Radiation chemical mechanisms of single- and double-strand break formation in irradiated SV40 DNA. Radiat Res 1991; 126: 251–9.
13. Siddiqi MA, Bothe E. Single- and double-strand break formation in DNA irradiated in aqueous solution: dependence on dose and OH radical scavenger concentration. Radiat Res 1987; 112: 449–63.
14. Ward JF. Some biochemical consequences of the spatial distribution of ionizing radiation-produced free radicals. Radiat Res 1981; 86: 185–95.
15. Kassis AI, Sahu SK, Kortylewicz ZP, Baranowska-Kortylewicz J, Adelstein SJ. DNA strand breaks in plasmid pBR322 following positional decay of I-125. J Nucl Med 1994; 35: 79P.
16. Neidle S. The molecular basis for the action of some DNA-binding drugs. Prog Med Chem 1979; 16: 151–221.
17. Pjura PE, Grzeskowiak K, Dickerson RE. Binding of Hoechst 33258 to the minor groove of B-DNA. J Mol Biol 1987; 197: 257–71.
18. Bathini Y, Rao KE, Shea RG, Lown JW. Molecular recognition between ligands and nucleic acids: novel pyridine- and benzoxazole-containing agents related to Hoechst 33258 that exhibit altered DNA sequence specificity deduced from footprinting analysis and spectroscopic studies. Chem Res Toxicol 1990; 3: 268–80.