Range of Radiochemical Damage to DNA with Decay of Iodine-125

Abstract. *Studies of the length of DNA fragments produced upon decay of iodine-125–labeled deoxycytidine that was located at a single position within a DNA fragment of defined sequence demonstrate that most radiochemical damage occurs within 15 to 20 angstroms of the site of iodine-125 decay. However, DNA strand breakage was detectable up to 70 angstroms from the site of iodine-125 decay.*

Decay of ^{125}I in the vicinity of DNA results in extensive radiochemical damage. Each decay of ^{125}I incorporated into DNA as iododeoxyuridine (IdU) results in a double-strand break (*1, 2*). Double-strand breaks are also produced by decay of ^{125}I-labeled aminoacridines that are noncovalently bound to DNA (*3, 4*). The cytocidal effects of ^{125}I incorporation into the DNA of bacteriophages (*1, 2*), *Escherichia coli* (*2, 5*), and mammalian cells (*6*) is presumably a consequence of DNA damage since lethality is correlated with the accumulated number of ^{125}I decays. For ^{125}I-IdU suicide in bacteriophages T1 and T4 (*1, 2*) and a *rec* A^- strain of *E. coli* (*5*), each lethal event corresponded approximately to a single ^{125}I decay. The cytotoxicity of ^{125}I-labeled aminoacridines in cultured mammalian cells (*4*), which is also probably a consequence of damage induced by ^{125}I decay, suggests that covalent linkage of ^{125}I to compounds such as hormones that bind specifically to the DNA of subpopulations of cells might provide a new class of selective cytotoxic agents of possible therapeutic value. An important consideration for the design of such reagents is the range of ^{125}I damage to DNA.

To measure the range of ^{125}I damage, we constructed DNA molecules that contained a single ^{125}I-labeled deoxycytidine (^{125}I-dC) at a known position within a DNA fragment of defined sequence. The DNA fragments were also labeled at one terminus with ^{32}P. The decay of ^{125}I produced strand breaks. The range of ^{125}I damage could be deduced by determination of the lengths of the ^{32}P-labeled cleavage products on high-resolution polyacrylamide gels. The design of the experiments permitted measurements of the range of ^{125}I-induced cleavage in the DNA strand to which the ^{125}I was covalently bound, as well as to the complementary strand of the double helix. The methods used here provide a general technique for the measurement of the range of radiochemical damage to macromolecules.

The use of DNA fragments of defined sequence permits construction of DNA molecules labeled at known positions. To determine the distribution of breaks that occur on the same strand to which a ^{125}I-labeled molecule is covalently bound, we constructed the DNA molecule shown in Fig. 1A. The 5′ end of one strand of a DNA fragment containing 59 nucleotides was labeled with ^{32}P. A single ^{125}I-dC residue was introduced on the same strand as the penultimate 3′ nucleotide (58 nucleotides away). The double-labeled DNA fragment was stored for 7 weeks to accumulate a sufficient number of ^{125}I decays for the analysis (the half-life of ^{125}I is 60 days). Serially diluted samples of the DNA were denatured and layered on a high-resolution urea–polyacrylamide gel to determine the length distribution of the labeled products.

An autoradiogram of such a gel illustrates the results of this experiment (Fig. 2). The length of the DNA products was determined by reference to the length of the DNA products produced by the Maxam-Gilbert DNA sequencing reactions (*7*) (lanes 1 to 4). Most of the radioactive substance migrated as a single species containing 59 nucleotides. We also observed DNA products that migrated faster than the 59-nucleotide product. The spacing between the shorter DNA products is equivalent to the spacing between adjacent nucleotides in the DNA sequencing tracks.

The 59-nucleotide DNA molecules contained both ^{32}P and ^{125}I, as determined by measurement of Cerenkov radiation and gamma-ray emission. The shorter DNA products contained only ^{32}P, as would be expected for scission products of ^{125}I decay. No DNA products shorter than the major product were observed on analysis of freshly prepared samples of similarly labeled DNA molecules.

The relative amounts of the shorter DNA fragments were determined by scanning the autoradiogram with a densitometer. The zero position on the abscis-

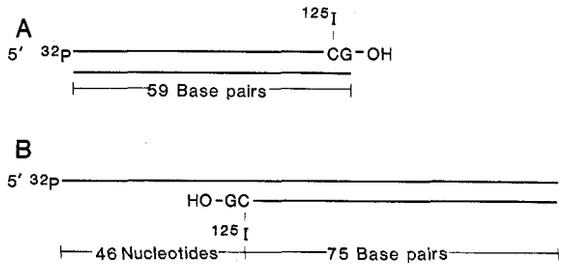
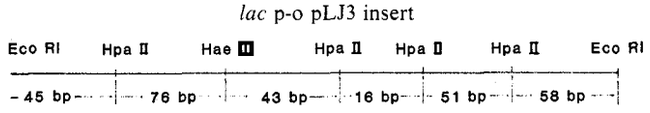


Fig. 1. Diagram of DNA molecules labeled at known positions with ^{125}I and ^{32}P. Deoxycytidine triphosphate (dCTP) labeled with ^{125}I (specific activity, 800 Ci/mmole) was supplied by New England Nuclear, Boston, Massachusetts. For 5′ end labeling, γ-^{32}P–labeled adenosine triphosphate (specific activity, 1500 Ci/mmole) was used (*9*). Deoxycytidine labeled with ^{125}I (specific activity, 800 Ci/mmole) was incorporated into DNA by filling out 3′ termini of Hpa II restriction fragments by incubation with ^{125}I-dCTP and unlabeled deoxyguanosine triphosphate. The substrates were constructed from regions of the *E. coli lac* promoter-operator (p-o) region that were contained in the pLJ3 plasmid. The construction, growth, purification, and isolation of pLJ3 plasmid DNA have been described (*10–12*).



(A) The DNA fragment that contained a ^{32}P end label in the 5′ position and a ^{125}I-dC residue on the same strand was prepared by digesting the 5′ end-labeled *lac* p-o insert of the pLJ3 plasmid with Hpa II and labeling with ^{125}I-dCTP as described above. A restriction fragment 59 nucleotides long was derived from the right end of the pLJ3 insert. (B) The DNA substrate that contained the 5′ terminal ^{32}P label and the ^{125}I-dC on opposite strands was constructed as follows. A single-stranded ^{32}P end-labeled DNA molecule was prepared by digestion of the ^{32}P end-labeled *lac* p-o insert of the pLJ3 plasmid with the restriction endonuclease Hae III to produce a fragment 121 nucleotides long. After denaturation, the end-labeled fragments were separated from the unlabeled complementary strands (that are four nucleotides shorter) by electrophoresis on a high-resolution denaturing polyacrylamide gel of the type used for DNA sequencing (*7*). The 121-nucleotide DNA strand labeled with ^{32}P at the 5′ end was derived from the left end of the *lac* p-o fragment. The ^{125}I-dC–containing strand of the double-labeled substrate was obtained by Hpa II digestion of the *lac* p-o insert and labeling with ^{125}I-dCTP as described above. The labeled fragments were separated on a 6 percent nondenaturing gel, and the largest fragment was recovered and further digested with Hae III. The larger of the two Hae III fragments, which corresponds to the 76-base-pair fragment of *lac* p-o restriction map, was isolated and is homologous to the 3′ portion of the 121-nucleotide single-stranded fragment labeled with ^{32}P at the 5′ end (nucleotides 45 to 121). The hybrid was formed by mixing the two components in approximately equimolar portions and annealing at 60°C. The hybrid product was separated from unhybridized DNA by electrophoresis on a 6 percent nondenaturing polyacrylamide gel.

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sa of Fig. 2B corresponds to a DNA molecule 57 nucleotides long, the length expected for cleavage of the phosphodiester bond immediately adjacent to the labeled ^{125}I-dC residue. More than 70 percent of the strand scission events occurred at a distance less than five nucleotides from the site of ^{125}I decay. Within this range, the frequency of strand scission declined sharply as a function of distance from the site of decay and then fell more gradually. Strand breaks are detectable at distances of 20 nucleotides or more from the site of ^{125}I decay.

The distribution of strand scission events on the DNA strand complementary to the strand that is labeled with ^{125}I-dC was determined for a partially double-stranded hybrid (Fig. 1B). The ^{125}I-dC residue of the hybrid was opposite the guanosine residue located 46 bases from the 5′ end of the ^{32}P-labeled strand. The hybrid was incubated for 44 days and analyzed as before. Fragments that correspond to scission events in the DNA strand complementary to the ^{125}I-dC–labeled strand were observed in the hybrid that was incubated for 44 days but not in a hybrid that was prepared shortly before the analysis. The size distribution of ^{32}P-labeled fragments produced in the experiment was further examined by densitometer scanning (Fig. 3). As before, the frequency of DNA strand scission events fell off rapidly within the first four to five nucleotides from the site of ^{125}I decay and then declined at a slower rate. Breaks on the strand opposite the site of ^{125}I decay could be detected at a distance of at least ten nucleotides. Similar results were obtained with a hybrid formed between a single-stranded 121-nucleotide fragment labeled with ^{32}P at the 5′ end and a DNA fragment complementary to residues 45 to 104 that contained a ^{125}I-dC residue opposite the guanosine at position 46 (not shown).

Our experiments provide data on the range of radiochemical damage to DNA after the decay of incorporated ^{125}I. Two zones of DNA breakage were observed. More than 70 percent of the observed strand breaks occurred within five nucleotides of the site of covalent attachment of the ^{125}I to the DNA. The frequency of strand scission events fell off rapidly with increasing distance within this zone on both the labeled and the complementary DNA strands. We estimated the radius of this zone to be 10 to 15 Å.

A displacement of the maximum number of labeled breakage products from the position of ^{125}I decay was observed for cleavage of both the labeled and complementary strand of DNA. The most likely explanation of this phenomenon is that each ^{125}I decay results in multiple breaks within the same molecule. Since only ^{32}P-labeled DNA fragments are detected, there would be an experimental bias toward measurement of the shorter DNA products. Multiple breaks per ^{125}I decay would also explain the asymmetric distribution of labeled DNA products around the site of ^{125}I

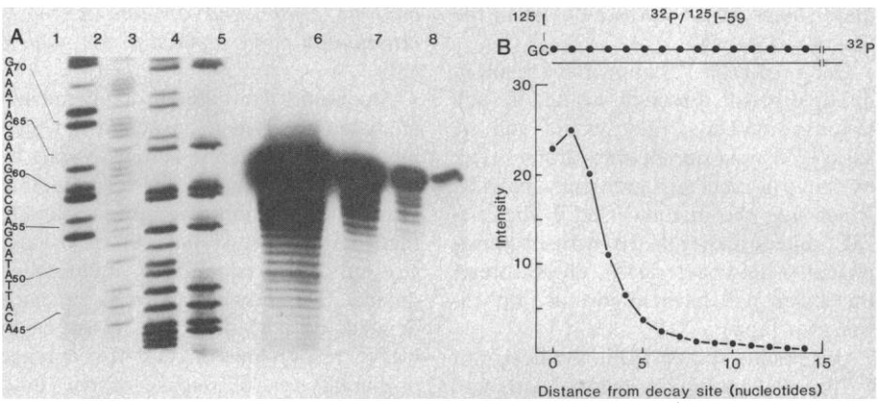

Fig. 2. (A) Breakage of DNA by ^{125}I decay of covalently bound ^{125}I. A sample of DNA molecules of the structure pictured in Fig. 1A was incubated in a gel slice that contained a 0.05*M* tris-borate buffer, *p*H 7.5, for 7 weeks at 10°C. It was then eluted from the gel, denatured, and layered on an 8 percent urea–polyacrylamide gel. Samples of a 169-nucleotide DNA molecule labeled with ^{32}P at the 5′ end (the right-end fragment of the end-labeled *lac* p-o insert) was subjected to the Maxam-Gilbert DNA sequencing reactions (*7*) and layered on adjacent lanes. The autoradiogram of the gel is shown. Lanes 1 to 4 are the DNA sequencing tracks (G, G + A, C + T, and C, respectively) (*7*). Lanes 5 to 8 contain serial dilutions of the double-labeled DNA molecule: lane 5, no dilution; lane 6, 1:5 dilution; lane 7, 1:25 dilution; and lane 8, 1:125 dilution. (B) The relative yield of DNA fragments of known length was determined by scanning the autoradiogram in (A) with a densitometer. Lanes 5, 6, and 7 were scanned with the same gain setting. Each scan consisted of an off-scale signal followed by a series of discrete peaks of decreasing intensity. Regions of overlap of the discrete peak regions of scans of adjacent lanes enabled calculation of scaling factors between scans of adjacent tracks, on the basis of peak height. When the entire process was repeated with a different gain setting, a similar composite was obtained. The peak at 0 corresponds to the mobility of the fragment that would result from elimination of the ^{125}I-dC nucleotide at a position 58 nucleotides from the labeled ^{32}P end. The intensity scale is in arbitrary units.

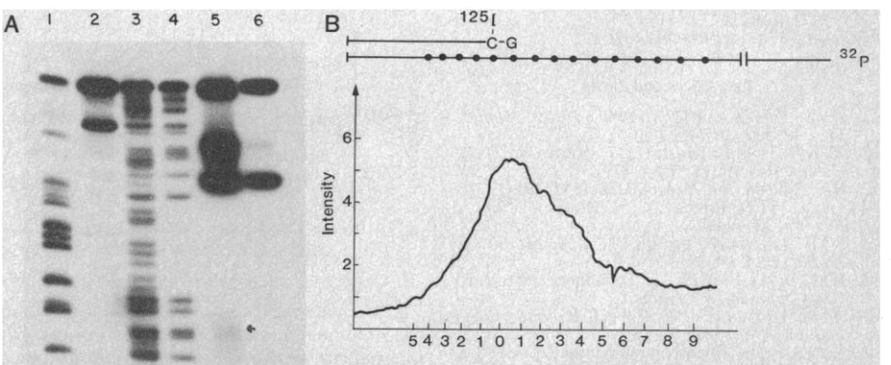

Fig. 3. (A) Breakage of DNA by decay of ^{125}I covalently bound to the complementary strand. A sample of DNA of the structure pictured in Fig. 1B was incubated for 44 days at 10°C in a buffer containing 0.3*M* NaCl, 20 m*M* tris-HCl, *p*H 7.4, and 4 m*M* Na_2EDTA. The DNA hybrid was denatured with heat and layered on an 8 percent urea–polyacrylamide gel (lane 5), together with similar hybrid prepared 5 days before electrophoresis (lane 6). The products of the Maxam-Gilbert DNA sequencing reactions (*7*) performed on the 121-nucleotide molecule labeled with ^{32}P at the 5′ end and a DNA fragment obtained by Hae III cleavage of the end-labeled *lac* p-o insert of the pLJ3 plasmid (the left fragment) were layered on the same gel. The reactions were G, G + A, C + T, and C, in lanes 1 to 4, respectively. The autoradiogram of the gel is shown. The arrow in lane 5 indicates the length of a DNA product that would be produced by elimination of the nucleotide opposite the ^{125}I-dC residue. (B) The relative amount of strand breakage was determined by a densitometer scan of lane 5. The 0 position on the abscissa indicates the product that would be produced by elimination of the guanosine nucleotide 46 nucleotides from the ^{32}P-labeled end terminus that is directly opposite the ^{125}I-dC residue. The intensity scale is in arbitrary units.

decay. However, in this case, bias for shorter strands might also reflect the asymmetric structure of the DNA molecule pictured in Fig. 1B. The single-stranded character of a portion of the hybrid might affect both the sensitivity of the DNA to radiochemical damage and the distance of the nucleotides from the site of ^{125}I decay.

Decay of each ^{125}I appears to result in multiple breaks on each strand. If such lesions do occur, they would still be assayed as just one double-strand break by neutral sucrose gradient analysis, which was the method used in the ^{125}I-IdU suicide experiments with bacteriophages (*1*) and bacteria (*5*). The nature of the lesion will have important implications for repair.

Our results should aid in the design of ^{125}I-labeled cytotoxic agents. Although the range of ^{125}I damage to DNA extends to 70 Å or more from the site of decay, for maximum efficiency the ^{125}I isotope should be located no more than 15 to 20 Å from the double helix. This limited range of damage following ^{125}I decay may also prove useful in studying the interaction of DNA with proteins and other ligands that can be labeled with ^{125}I. The results of these experiments are consistent with the calculated energy deposition of low-energy electrons released in the vicinity of I^{125} decay (*8*).

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Fluctuating Responses at a Central Synapse: *n* of Binomial Fit Predicts Number of Stained Presynaptic Boutons

Abstract. *Binomial predictions provided a better description than the Poisson law of fluctuating unitary inhibitory postsynaptic potentials evoked in the goldfish Mauthner cell by impulses in presynaptic interneurons. The number of terminal boutons established on this target cell by each horseradish peroxidase–filled interneuron corresponded to the value of the binomial parameter* n.

Amplitude fluctuations of chemically mediated postsynaptic potentials evoked by single presynaptic impulses can be described by mathematical relations that share one common assumption, namely that the response is made up of an integral number of equal basic units, called quanta. The number of units released depends on both the total amount capable of responding, n, and the average probability, p, of release of one unit; then m, the mean number of quanta responding to one impulse (mean quantal content), can be expressed by the product np. When p is made small, such as at the frog neuromuscular junction bathed in low Ca^{2+}, high Mg^{2+} solution, the fluctuations are described by Poisson's law, $p_k = (m^k/k!)\, e^{-m}$, where k is a particular number of units constituting a response (*1*). In this case n is large, and it was thus postulated to represent the population of presynaptic vesicles (*2*). However, it is difficult with this model to give independent meaning to n and p. Furthermore, at most junctions studied thus far, especially when pharmacological manipulations were avoided, the binomi-

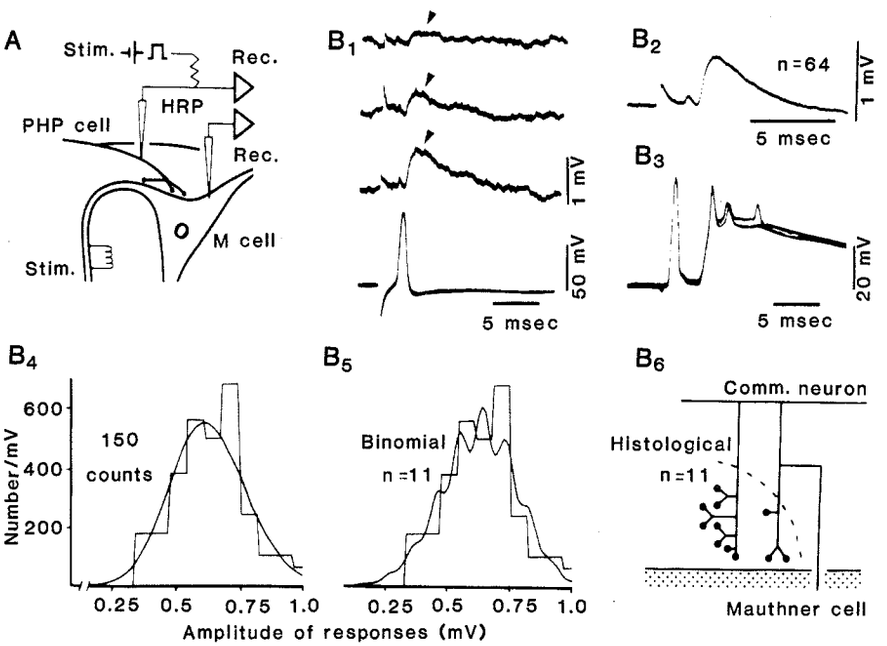

Fig. 1. Evidence for quantal fluctuations of unitary IPSP's. (A) Experimental arrangement used for simultaneous intracellular recordings (*Rec.*) from the M cell and a presynaptic inhibitory interneuron (*PHP cell*), both neurons being identified by their characteristic responses to antidromic stimulation (*Stim.*) of the M axon in the spinal cord. The presynaptic electrode was also used for intracellular stimulation (*Stim.*) and subsequent staining with HRP. (B_1 to B_5) Properties of depolarizing IPSP's recorded in a Cl^--injected M cell throughout the same experiment. (B_1) Variable amplitude of unitary IPSP's (arrows, upper three traces) following single presynaptic impulses directly evoked at a frequency of 1 per second. Only one spike is shown (lower trace). (B_2) Computer-averaged unitary IPSP ($N = 64$). (B_3) The maximum amplitude IPSP following antidromic activation of the recurrent collateral network was large enough to fire the M cell. (B_4 and B_5) Comparisons of observed IPSP amplitude variations (stepwise distributions for 150 responses) with best fits (continuous curves) obtained with a Poisson (B_4) or binomial (B_5) relation. Abscissas show the amplitude of responses evoked by nerve impulses; ordinates, the density of observations expressed as number of occurrences per millivolt. The likelihood criterion was better for the binomial, which yielded a value of 11 for parameter n, while p and q were 0.62 and 80 μV, respectively (when derived from the Poisson model, m and q were, respectively, 19 and 30 μV). The binomial prediction satisfied the Kolmogorov test ($P > .05$) but the Poisson did not ($P < .01$). (B_6) Schematic representation of the reconstructed presynaptic cell's terminals in relation to the M cell body (shaded area) and its axon cap (dashed line). This cell, which was a commissural (*Comm.*) neuron, established 11 synaptic boutons, represented by closed circles.

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