Radiation Target Analyses of DNA Template/Primer Complexes

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ABSTRACT Frozen solutions of low molecular weight DNA template/primer complexes, in the absence and presence of HIV-1 reverse transcriptase, were irradiated with high-energy electrons. Molecules that survived the radiation exposure were quantified and analyzed using radiation target theory. Transfer of radiation-deposited energy was observed by the damage caused. It was found that damage (as a polynucleotide chain break) was observed in one chain when the radiation interaction occurred in the other chain, suggesting a transfer of energy. In contrast, the target sizes of the DNA template/primers were not altered if bound to HIV-1 reverse transcriptase, signifying that the deposited radiation energy is not transferred between protein and nucleic acid.

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The effects of ionizing radiation on DNA have been widely studied for many years (1), although radiation target analyses had only been reported before well-defined preparations and advanced techniques became available. Recently it was demonstrated that radiation damage in RNA ribozymes occurred only locally in the molecule (2), which is in sharp contrast with the well-established spread of radiation damage throughout polypeptides (3,4). This surprising result stimulated questions about the nature of radiation damage in other nucleic acid complexes, in particular DNA. In this study, radiation analyses of defined DNA template/primer (T/P) complexes were conducted in the absence and presence of HIV-1 reverse transcriptase (RT), an enzyme that binds these nucleic acid complexes with high affinity and previously has been studied by radiation target analysis (5).

Two different DNA T/P complexes were subjected to radiation inactivation analyses. One was a homopolymeric poly$(C)_{30}$-poly$(G)_{18}$ T/P (14.4 kDa) that consisted of an 18-nucleotide homopolymeric primer (dG_{18}; 5865 Da) annealed to a 30-nucleotide homopolymeric template (dC_{30}; 8615 Da). The other was a heteropolymeric T/P (designated $(T)_{30}$-$(P)_{18}$; 15.8 kDa) that consisted of an 18-nucleotide heteropolymeric primer (5′-CCTGTTCGGGCGCCACTG-3′; 5756.6 Da) annealed to a complementary 30-nucleotide heteropolymeric template (5′-GAAAATCTCTAGCAGTGGCGCCCGAACAGG-3′; 10089.2 Da). All DNA oligomers were synthesized and HPLC-purified by Integrated DNA Technologies (Coralville, IA). DNA T/P complexes were formed by combining equimolar concentrations of template and primer at 90°C and allowing the mixture to slowly cool to ambient room temperature. Samples for irradiation were diluted to 2 mg/ml in 20 mM Tris-HCl (pH 7.5, 20°C) and 100 mM NaCl. This concentration of macromolecule has previously been shown to be high enough to yield meaningful radiation target sizes and avert the need for free radical scavengers (6). Radiation exposures of 0–100 Mrads were obtained as described (7) from 10 MeV electrons produced by linear accelerators at the Armed Forces Radiobiology Research Institute or at the National Institute of Standards and Technology. Following irradiation, the surviving double-stranded DNA species in the poly$(C)_{30}$-poly$(G)_{18}$ samples were quantified using the Quant-iT PicoGreen double-stranded DNA quantitation assay (Molecular Probes, Invitrogen, Carlsbad, CA). This assay can selectively detect as little as 25 pg/mL of double-stranded DNA in the presence of single-stranded DNA, RNA, and free nucleotides and is linear over three orders of magnitude with little sequence dependence. The template and primer strands of the $(T)_{30}$-$(P)_{18}$ heteropolymeric T/P complex were analyzed independently using denaturing polyacrylamide gel electrophoresis to separate the strands, and SYBR-Gold reagent (Molecular Probes, Invitrogen) to detect the oligonucleotides. The radiation inactivation profiles of both DNA T/P complexes were identical, whether measured as total double-stranded DNA or individually as the template and primer oligonucleotides (Fig. 1). In all cases the observed target sizes were ~16,000 Da (Table 1), which corresponds to their double-stranded mass. Theoretical inactivation curves for a 5-kDa and a 10-kDa target clearly do not fit the data (Fig. 1).

To ascertain whether DNA-protein interactions impacted on the target sizes of the DNA T/P complexes, irradiation experiments were also carried out in which both T/P complexes were bound to HIV-1 RT. HIV-1 RT is a 117-kDa heterodimeric enzyme that consists of 66 kDa (p66) and 51 kDa (p51) subunits. The enzyme is multifunctional and exhibits RNA-dependent DNA polymerase (RDDP), DNA-dependent DNA polymerase, and ribonuclease H activities. Previous

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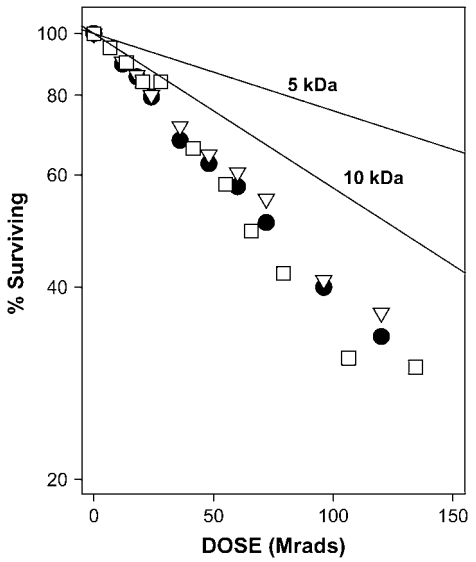

FIGURE 1 Surviving DNA in irradiated T/P samples after exposure to various doses of ionizing radiation at −135°C. Total DNA from poly(C)$_{30}$-poly(G)$_{18}$ (*squares*), template DNA (*triangles*), and primer DNA (*circles*). Data are from three independent experiments. Solid lines show theoretical survival of a 5-kDa and a 10-kDa molecule.

studies have demonstrated that both the structural and functional target sizes of HIV-1 RT correspond to that of the heterodimeric protein (5). The radiation target sizes of HIV-1 are also unaffected by the presence of DNA T/P complexes (5). In this study, recombinant HIV-1 RT was purified according to published techniques (8,9) and the target sizes of the enzyme were determined by SDS-gel electrophoresis and by analysis of the residual RDDP activity, as described previously (5). The radiation inactivation profiles of HIV-1 RT determined by SDS-PAGE and RDDP activity in this study (Table 2 and Fig. 2) correlate with the heterodimeric structure of the enzyme and are essentially identical to those determined previously (5). Therefore this enzyme acted as an internal control for the new study. As described above, the irradiated samples were also analyzed for surviving DNA T/P complexes. The observed target sizes for both T/P complexes did not appear to be influenced by their interactions with HIV-1 RT (Table 2).

Taken together, these results demonstrate that the radiation-sensitive ''target'' mass of small double-stranded DNA T/P complexes, either alone or in association with HIV-1 RT, yielded consistent results irrespective of the method of measurement. Target sizes calculated for the DNA T/P complex or for each of the individual template and primer strands all yielded the same radiation target size of ~16 kDa, with very small error. Thus, a primary ionization in any single chain of DNA results ultimately in cleavage of another polynucleotide chain to which it is basepaired.

TABLE 1 DNA target sizes (in kDa) from multiple irradiated T/P preparations

poly(C)$_{30}$-poly(G)$_{18}$	15.9
*Heteropolymeric primer strand	18.1; 15.4
*Heteropolymeric template strand	17.1; 16.7
Average ± SD	16.7 ± 1.1

*Target sizes derived from two independent experiments.

TABLE 2 Target sizes (in kDa) from multiple independent irradiations of HIV-1 RT-T/P preparations

Target sizes determined for HIV-1 RT		
RDDP activity	104 ± 18	*91.5
p66 Subunit	102 ± 17	*93.9
p51 Subunit	100 ± 9	*95.0
Target sizes determined for T/P		
poly(C)$_{30}$-poly(G)$_{18}$		15.5
†Heteropolymeric primer strand		15.3;16.3
†Heteropolymeric template strand		16.5; 19.3
Average ± SD		16.4 ± 1.3

*Values taken from Sluis-Cremer et al. (5).
†Target sizes derived from two independent experiments.

As described previously (5), the radiation target sizes of HIV-1 RT were unaffected by the presence of DNA T/P complexes, indicating the absence of any transfer to the protein of radiation energy deposited in the DNA. These results clearly demonstrate the lack of energy transfer in the opposite direction. The crystal structure of an RT-T/P complex (10) shows that the principal contacts between the RT protein and T/P are via the deoxyribose sugar ring. Previous radiation studies of oligosaccharides (11), RNA ribozymes (2), and large DNA plasmids (T. J. Anchordoquy, M. d. C. Molina, and E. S. Kempner, unpublished data) suggested that the

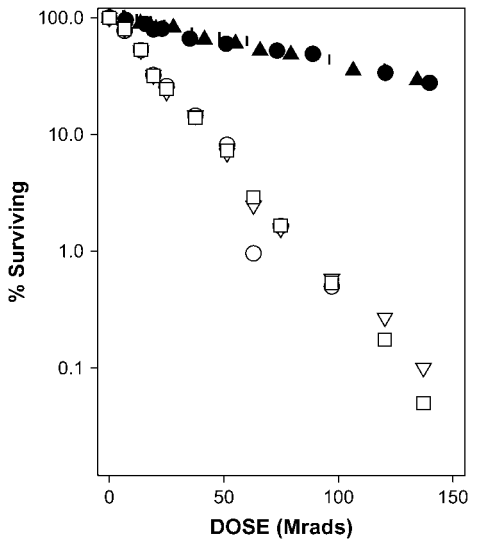

FIGURE 2 Surviving properties of irradiated HIV-1 RT-T/P after exposure to various doses of ionizing radiation at −135°C. RDDP activity (*circles*), p66 subunit (*squares*), p51 subunit (*triangles*), total DNA from poly(C)$_{30}$-poly(G)$_{18}$ (*solid circles*), template DNA (*solid triangles*), and primer DNA (*vertical lines*). Data from three independent experiments.

transfer of radiation-deposited energy was prevented by the presence of sugar rings in the polymer. This phenomenon could explain the isolation of radiation damage to RT from T/P and vice versa. In this case, the blockage of energy transfer from a polynucleotide may be due to the deoxyribose that is noncovalently bound to a polypeptide. A brief report of electron spin resonance signals in irradiated chromatin (13) indicated electron transfer, but not electron hole transfer, from histone into DNA. The association between histone and DNA is principally through noncovalent interactions with DNA phosphate groups (14). The conflicting results from irradiated RT-T/P and chromatin could be explained by the difference in sugar ring interactions.

The T/P inactivation curve appears to be a simple exponential, indicating only a single-sized target. Therefore the efficiency of energy transfer between DNA strands must approach 100%. If there were subsets of T/P complexes that did not permit energy transfer, the inactivation curve would not be a simple exponential but rather the sum of the curves from the individual subsets (15). This theoretical model does not fit the data at all.

Previous studies of radiation effects on DNA and of energy transfer between strands (1) were principally assayed by ''double-strand breaks''. These are cleavages of both polymer backbones where the breaks are within a very few base-pairs of each other. These differ from polymer scissions detected in this study in which there is no restriction as to the relative positions of the two breaks. The principal conclusions of this study, therefore, is that in these particular T/P DNA complexes, when exposed in the frozen state to ionizing radiation, a single radiation interaction in either strand results in scission of that strand as well as a scission somewhere in the opposite strand. No damage is detected in either the protein RT or the T/P when the other component is damaged.

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