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Specificity of ionizing radiation-induced mutagenesis in the *lac* region of single-stranded phage M13 mp10 DNA

Hitoshi Ayaki, Ken-ichi Higo and Osamu Yamamoto

Research Institute for Nuclear Medicine and Biology, Hiroshima University, 2-3, Kasumi 1-chome, Minami-ku, Hiroshima 734, Japan

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ABSTRACT

M13 mp10 single-stranded phage DNA was irradiated with ^{60}Co gamma-rays, and transfected into Escherichia coli. One hundred and sixteen mutant clones having lesions in the lac insert were selected, and mutational sites were examined by DNA sequence analysis. Fourteen out of the 15 nucleotide changes thus detected were base substitutions, and the rest was a base addition. Transitions and transversions were almost equal in number. Mutational events were observed at cytosine residues more frequently than at other residues, and the predominant base change was a C → T transition. Possible roles in gamma-ray-induced mutagenesis played by the misincorporation of dAMP owing to radiolytic derivatives of cytosine residues and/or formation of apurinic/apyrimidinic sites are discussed.

INTRODUCTION

Exposure of DNA to ionizing radiation may result in various types of damages, e.g., strand breaks, base modifications, and production of apurinic/apyrimidinic (AP) sites, which change coding properties of the DNA. Previous investigations on the gamma-ray-induced mutational spectrum with lac I system (1,2), His^+ reversion system (3), or M13 phage amber mutation reversion system (4), showed no specificity as to the target bases. However, targeted DNA regions in these systems were very limited.

Recently developed direct DNA sequencing technique now permit us to detect nucleotide changes rapidly. Using these technique, base substitution specificities of chemical mutagens (5-7) and of UV-irradiation (8-11) were studied. In order to examine more closely the mutational specificity of gamma-ray irradiation, we applied the direct nucleotide sequencing technique to M13 mp10 hybrid phage DNA, which contains the lac regulatory genes and a part of the lac Z structural gene of

Escherichia coli. It is possible to induce mutations at many sites in the lac region, which is not essential for the phage growth, and the mutants can be selected by plaque color on a indicator medium.

MATERIALS AND METHODS

Chemicals and Enzyme. Unlabeled nucleotides, [^{35}S](α-thio)dATP, and M13 sequencing primer were purchased from Amersham, England. 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) and isopropyl-β-D-thiogalactopyranoside (IPTG) were obtained from Boehringer Mannheim, West Germany. E. coli DNA polymerase I large fragment (Klenow fragment) was from Takara Shuzo, Japan.

DNA preparation. Bacteriophage M13 mp10 was grown in E. coli K12 JM101, and single-stranded (ss) phage DNA was isolated as described by Messing (12).

Irradiation. M13 mp10 ss DNA (50 μg/ml) in 10 mM Tris-HCl (pH 7.5)/ 1 mM EDTA was irradiated with ^{60}Co gamma-rays at a dose rate of 100 Gy (10 krad)/min at 0°C.

Transfection and Plating. E. coli JM101 was grown to a density of 0.3 OD (550 nm) in YT medium consisted of Bactotryptone (8 g/l), Yeast extract (5 g/l), and NaCl (2.5 g/l). Cells were resuspended in 50 mM $CaCl_2$, incubated at 0°C for 30 min with irradiated phage DNA, and heat-shocked at 37°C for 5 min. This mixture was then added to 3 ml of molten YT medium with 0.6 % agar containing Xgal (0.8 mg), IPTG (0.95 mg), and exponentially growing JM101 (0.2 ml), and poured on YT medium plate. The plate was incubated overnight at 37°C.

DNA sequencing. DNA sequencing was carried out according to the chain termination method (13) with the modifications by Messing (12). Using a 17 base synthetic primer (5'-GTAAAACGACGGCCAGT-3'), about 200 bases in the lac region were determined.

RESULTS AND DISCUSSION

Mutagenesis of M13 mp10 single-stranded phage DNA by gamma-rays. Transfecting activity of the irradiated M13 mp10 ss DNA decreased exponentially with increasing radiation dose. Phage DNA treated with 200 Gy resulted in 0.1 % survival. The mutation frequency increased to 2.5×10^{-3}, about 5 times that of unirradiated DNA.

Table 1. Types of gamma-ray-induced mutations

Types of mutations	Number of occurrence	%
Base substitutions	14	93
Transition	7	46
Transversion	6	40
Nontandem double	1	7
Frameshifts	1	7
Deletion	0	
Addition	1	7
Totals	15	100

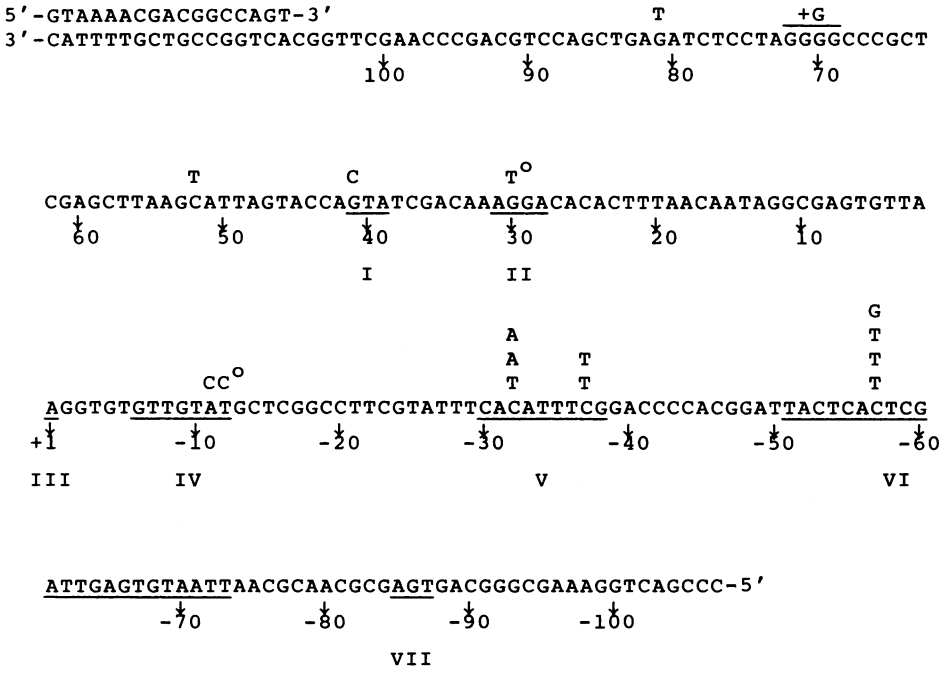

Figure 1. Nucleotide sequence of the part of wild type β-galactosidase gene and mutational sites of the mutants. 5'-GTAA AACGACGGCCAGT-3' is a synthetic primer for DNA sequencing. Base changes are shown above the wild type sequence in capital. Addition and nontandem double mutation are indicated by + and o, respectively. I-VII are shown as follows: I, translation start codon; II, ribosome binding site (15); III, transcription start site (16); IV, Pribnow box (17); V, -35 region (17); VI, CAP binding site (18); VII, lac I termination codon.

Sequence analysis of gamma-induced mutagenesis. One hundred and sixteen colorless or light-blue mutants were isolated and sequenced, as described in Materials and Methods. Nucleotides changes were detected in 15 clones (approximately 13%). The remaining mutant clones probably have base changes outside of the regions we sequenced. Types of mutations are summarized in Table 1. Single base substitutions represented a large part of the total induced mutations (13/15, 87%). Other mutations were a nontandem double base substitution and an addition, no deletion being observed. Transversions and transitions occurred with about equal frequencies, although a preference for transitions in the spontaneous mutational spectrum of M13 mp2 was reported recently (14).

The distribution of the base changes is shown in Figure 1. Base substitutions were found at nine sites. Mutations at three sites (positions -32, -37,and -57) were observed more frequently than at other sites. The apparent preference for position -57 was also shown in the UV-induced mutagenesis of M13 mp2 phage (10). The "hotspots" in the -35 region in the mutations induced by UV-irradiation (10) and by depurination (14) of M13 mp2, differing from those shown here, have been also reported.

Table 2 shows the specificity of the gamma-ray-induced base substitutions. All the transitions were C → T base changes except for one substitution (T → C). Mutations involving cytosine residues (in the template strand) constitute about 70% of all the base changes observed. The selectivity for cytosine residues calls for examination of a wider range of mutants than available here.

If all the base changes shown in Table 2 are the consequences of misincorporation of dNMP opposite the damaged nucleotides during the first round of replication in the host cell, the apparent preference for incorporation are as follows: adenine residue (60%), guanine residue (20%), thymine residue (13%), cytosine residue (7%).

Possible mechanism of gamma-induced mutagenesis. Heat treatment of poly (dC) induced the formation of uracil residues by deamination of cytosine residues. When the heat-treated poly (dC) was used as the template for DNA replication by DNA polymerase I,

Table 2. Specificity of base substitutions induced by gamma-rays

Template base	Mutational sites	Types of mutations		Number of occurrence*
		Transition	Transversion	
G	3	G → A		0
			G → T	2
			G → C	1
A	1	A → G		0
			A → T	0
			A → C	1
T	1	T → C		1
			T → G	0
			T → A	0
C	4	C → T		7
			C → G	1
			C → A	2

* Nontandem double-base substitution is included.

dAMP incorporation was observed (19). The predominance of C → T transitions observed in the present study may also reflect the modification by gamma-irradiation from cytosine to its derivatives (20,21) especially such as 5,6-dihydroxy-5,6-dihydro uracil (22,23), which have same hydrogen-bonding capacity as thymine residues. This would also result in C → T transition.

When uracil residues were excised from the heat-treated poly (dC) by an enzyme to form apyrimidinic sites, dAMP was preferentially incorporated, as compared with dTMP, opposite the abasic site (19). Similar preferential incorporation of dAMP *in vivo* have been reported for heat-induced apurinic sites (14) and for neocarzinostatin-induced apyrimidinic sites at cytosine residues of lambda *cI* mutants (24). The formation of AP sites by ionizing radiation has been already established, e.g., 0.06 base released per 100 eV (25). Our finding that dAMP is most preferentially incorporated opposite the damaged nucleotide suggests that AP site formation also play a role in ionizing-radiation mutagenesis. Other types of base substitutions may also reflect the order of the preference of misincorporation at AP sites (see Discussion in ref. 14). Whether the mutational spectrum in cells active in "SOS repair reactions" differs from that in SOS-noninduced cells (present study) remains to be seen.

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