STUDY OF LETHAL AND MUTAGENIC EFFECTS OF ^{3}H-DECAY IN VARIOUS POSITIONS OF DNA ON A MODEL OF EXTRACELLULAR LAMBDA PHAGE

L. V. Konevega, V. L. Kalinin

There are 10 definite non-exchangeable positions in DNA (7 in 4 nucleic bases and 3 in deoxyribose moieties) where ^{3}H incorporated instead of ^{1}H remains for a long time, up to its nuclear conversion into ^{3}He. ^{3}H transmutation yields molecular products of ^{3}H substitutions for hydroxyl residues on nucleic bases and deoxyribose. These products being stable analogs of natural components are retained in DNA structure in absence of enzymatic processes. Therefore the analysis of lethal and mutagenic consequences of ^{3}H decay gives a unique possibility to evaluate separately biological consequences of 10 different ^{3}H->OH substitutions in DNA. The most important lesions are 5-hydroxymethyl-U, 6-hydroxy-T, 5-hydroxy-C, 6-hydroxy-C, 8-hydroxy-A, 8-hydroxy-G and 2-hydroxy-A residues which in various combinations are induced also by both oxidative agents and ionizing radiation. We are presenting results of a systematic study of the lethal and mutagenic effects of ^{3}H decay after incorporation into 7 of the 10 possible non-exchangeable positions into DNA of extracellular lambda phage. The data concerning biological consequences of ^{3}H decay in deoxyribose residues of DNA were absent in literature.

We registered induction of the forward *c*-mutations in extracellular λ phage. This system has several advantages in comparison with cellular systems used by other authors. In this system all radiation and chemical processes accompanying ^{3}H decay are taking place in vitro and their products are unaccesible to cellular enzymes during expositions (up to 2 or more months). Only after the completion of these processes, labelled phage DNA is introduced into bacterial host cells where rapid fixation of genetic changes becomes possible. Hence for the first time we were able to separate mutagenic effects caused by replication errors (in the cells with uninduced SOS-repair system) and by error-prone mutagenic SOS-repair (after preliminary induction of SOS system by UV-irradiation). Our data are summarized in Table.

The following conclusions can be drawn from these data.

1. ^{3}H decay in 2' position of deoxyribose residues is accompanied by minimal lethal and mutagenic effects. This minimal level of genetic effects can be ascribed to nonspecific (radiation) action of β-particles of nuclear decay which is common to all positions of ^{3}H incorporation.

2. Lethal and mutagenic effects of ^{3}H decay within nucleic bases are 2 - 100 times higher than this basal "radiation-induced" level. Therefore, transmutation component of ^{3}H decay in all of these positions is biologically significant.

3. A considerable increase of the c-mutation frequency registered in unirradiated cells after ^{3}H decay in positions 5 and 6 of C and T residues and in position 8 of purine residues suggests that most probable products of ^{3}H->OH substitutions in these positions of nucleic bases are capable to complementary mispairing during normal DNA replication. This effect is most pronounced after ^{3}H decay in position 5 of C. It has been assumed earlier that in this case premutational DNA lesion is uracil which is generated by spontaneous deamination of the primary product - 5-hydroxy-C. However, *ung* mutation inactivating uracil-DNA-glycosylase which is capable to eliminate U from DNA does not diminish level of *c*-mutations.

Table

Lethal and mutagenic effects of ^{3}H incorporated into λ DNA, as measured on *E. coli* strain with wild-type repair genotype

^{3}H-precursor	Mutagencic efficiency (E_m) on: unirradiated cells	UV-irradiated cells	Efficiency of lethal effect (α)	Ref.
5–^{3}H-thymine	$(4.4 \pm 0.5)10^{-5}$	$(4.7 \pm 0.8)10^{-5}$	0.14 ± 0.01	[1]
6–^{3}H-thymine	$(2.4 \pm 0.3)10^{-5}$	$(3.6 \pm 0.6)10^{-5}$	0.16 ± 0.02	[3]
5–^{3}H-cytosine	$(6.5 \pm 0.6)10^{-4}$($)	$6.1 \cdot 10^{-4}$($)	0.14 ± 0.04	[2]
6–^{3}H-cytosine	$(4.4 \pm 0.3)10^{-5}$	$(20 \pm 2)10^{-5}$	0.13 ± 0.03	[3]
8–^{3}H-ade/gua	$(2.9 \pm 0.2)10^{-5}$	$(11 \pm 1)10^{-5}$	0.14 ± 0.01	[5]
(2′–^{3}H)dA/dG	$(6.7 \pm 0.6)10^{-6}$	$(3.6 \pm 0.4)10^{-5}$	$0.07 \pm 0,01$	[4]
(2′–^{3}H)dT	$(6,2 \pm 0.8)10^{-6}$	$(1.1 \pm 0.3)10^{-5}$	0.07 ± 0.01	[4]

α – number of lethal hits per ^{3}H decay.
E_M – increment of mutation frequency per ^{3}H decay.
($) – the same values were measured on *E. coli ung*$^-$ mutant.

Therefore, this hypothesis can be completely rejected.

^{3}H-decay in position 5 of thymine residues is also accompanied by significant induction of mutations. This effect was unexpected: 5-hydroxymethyl-U is a natural DNA component in some bacteriophages and was supposed to be of no biological consequences. Two peculiarities of this modified base can contribute to its mutagenic effect: 1) H–>OH substitution in methyl group of T is making hydrophylic this hydrophobic group and could in principle decrease its fidelity during complementary base pairing; 2) normal bacterial cells are lacking 5-hydroxymethyluracil-DNA-glycosylase discovered in mammalian cells and are incapable to repair this mispairing lesion.

On the contrary, minimal (among nucleic bases) mutagenic efficiencies of ^{3}H-decay in positions 6 of T and 8 of purines are probably determined by their efficient repair. Thymine residues, damaged by saturation of double bond or ring fragmentation, are eliminated by exonuclease III, and purine residues, modified by ^{3}H decay in position 8, can be repaired by formamidopyrimidine-DNA-glycosylase.

4. Inducible SOS-repair system contributes significantly to mutagenic effect of every position of ^{3}H incorporation, the only exception being position 5 of C where this contribution is probably masked by very high level of SOS-independent mutagenesis. However, the levels of SOS-effects are not equal for all positions of ^{3}H incorporation. SOS repair enhances the frequency of induced mutations 4 – 5 fold when ^{3}H decay damages A, G, or C residues or linked deoxyribose residues but its effect is unsignificant (no more than 1.5-fold) after modification of T and associated deoxyribose. These data confirm general "A rule" of error-prone misincorporation. According to this rule, repair of non-pairing DNA lesions is accompanied by preferential insertion of A residues. SOS-repair of these lesions on T residues cannot cause mutations due to reinsertion of the initial A:T base pair. In all other cases, SOS-repair has mutagenic consequences.

REFERENCES

1. *Konevega L.V., Kalinin V.L.* //Radiobiology, 1984. V.24. P.167.
2. *Konevega L.V., Kalinin V.L.* //Radiobiology, 1985. V.25. P.319.
3. *Konevega L.V., Kalinin V.L.* //Radiobiology, 1989. V.29. P.17.
4. *Konevega L.V., Kalinin V.L.* //Radiobiology, 1992. V.32. P.194.
5. *Konevega L.V.* //10th Int.Congress of Rad.Res., 1995. V.1. P.161.

PROPERTIES OF CLONED DOMINANT Gam18 ALLELE

V. L. Kalinin, V. N. Verbenko,
L. V. Kuznetsova, E. P. Krupjan

Mutant alleles Gam^r capable to enhance radiation-resistance of *E. coli* wild-type cells were cloned from the Gam^{r}444 mutant in a plasmid mini-Mu vector [1]. After introduction of these pGam^r plasmids into *recA13* or *rpoH15* mutants, high level of radiation-resistance was not obtained. Therefore expression of the cloned dominant gam^r mutationsl depends on *SOS* and *HSP* regulons.

Null mutation in chromosomal gene corresponding to *gam^{r}18* allele was constructed. Random insertions of mini-Tn10-Km^r transposon [2] into pGam18 were obtained by hop-transposition from λ1105 vector and a plasmid pGam^{s}18::Km^r (Cm^r), which had lost ability to transfer Gam^r phenotype and carries a *gam^{s}18*::Km^r insertion in the cloned Gam^r gene, was selected. Very slowly growing Km^{r}Cm^s transformants were selected after transformation of *recBC sbcB* recipient with pGam^{s}18::Km^r (Cm^r) plasmid DNA cut with EcoRI endonuclease. They are extremely sensitive to γ-rays and presumably are carrying chromosomal insertion *gam^{s}18*::Km^r. The behavior of this mutation in *recBC sbcB* background is typical for mutations in the *RecF* pathway.

Plasmid pGam18 restores radioresistance of *recBC sbcB gam^{s}18*::Km^r mutant and partly compensates defect in *uvrD* gene (Table 1), i.e. *gam^{r}18* mutation may be concerned with a helicase involved in repair and recombinant process. Transduction of *Kan* marker by Kohara phages [3] revealed homology of plasmid-borne allele with some helicases which had 3'→5' polarity (Table 2).

Thus, the dominant mutation *gam^{r}18* is related to RecF pathway of repair and recombination and mutant protein can be a reparative helicase.