RESPONSE OF THE AMES TEST TO DIFFERENT TYPES OF IONIZING RADIATION

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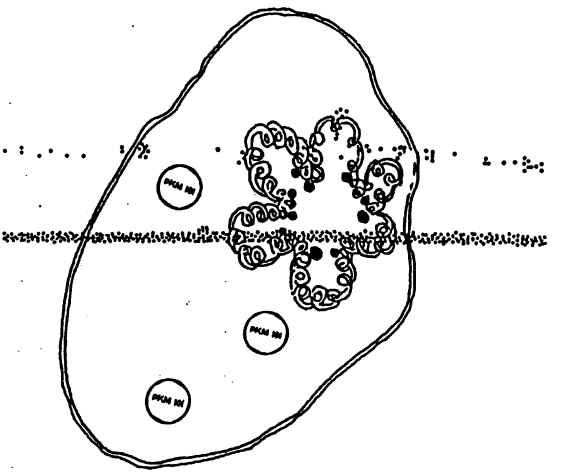

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INTRODUCTION

Thirteen years after the statement of B.N.Ames that x-rays and fission neutrons are able to induce reversions in different Salmonella tester strains, Isildar and Bakale (1) published a broad study concerning radiation induced mutagenicity and lethality in Ames tester strains with basepair substitutions (TA100, TA1535) and frameshift mutants (TA98, TA1538, TA2637, TA1537). Their study was performed with ^{60}Co-γ rays at a dose rate of 18 Gy/min.

Isildar and Bakale, did not perform experiments with Salmonellae containing the histidine requiring mutation hisG428, which is known to be very sensitive to oxidative mutagens. This and our new technique of delayed irradiation motivated the present studies.

MATERIAL AND METHODS

The following Salmonella strains were used in our study. All strains were kindly provided by Dr.B.N. Ames, Berkeley.

strain	genotype	type of mutation	mutagen-suscept. sequence	plasmid
TA2638	hisG428/rfa	bps	A/T	pKM101
TA104	hisG428/rfa/uvrB	bps	A/T	pKM101
TA2640	hisG428/rfa	bps	A/T	-
TA100	hisG46/rfa/uvrB	bps	G/C	pKM101
TA1535	hisG46/rfa/uvrB	bps	G/C	-
TA98	hisD3052/rfa/uvrB	fs	GCGCGCGC	pKM101
TA1538	hisD3052/rfa/uvrB	fs	GCGCGCGC	-
TA2637	hisC3076/rfa/uvrB	fs	(GGG)	pKM101
TA1537	hisC3076/rfa/uvrB	fs	(GGG)	-

bps = base substitution mutants
fs = histidine requiring frameshift mutants
rfa = deep rough mutant
uvrB = UV-excision repair deficient
(GGG) = the sequences surrounding the hisC3076 mutation ars not known, but the mutation is assumed to be a +G/C base pair in a sequence of G/C.

For mutagenicity experiments 0.1ml of a culture with about $1\text{-}5*10^9$ bacteria/ml were mixed with histidine and biotine supplemented top agar and were poured onto minimal glucose agar plates. For the determination of the viable fraction, diluted bacteria were mixed with 2ml top agar and were spread on nutrient agar plates. The protocol of Maron and Ames was adopted (3).

RESULTS

a) REVERSION TO HISTIDINE PROTOTROPHY

The technique of delayed irradiation has been utilized instead of the conventional irradiation briefly after plating. It leads to a substantially enhanced ratio of induced to spontaneous reversions (2). The advantages of the delayed exposure method are brought out in a comparison of the present results with the data earlier obtained by Isildar and Bakale (1).

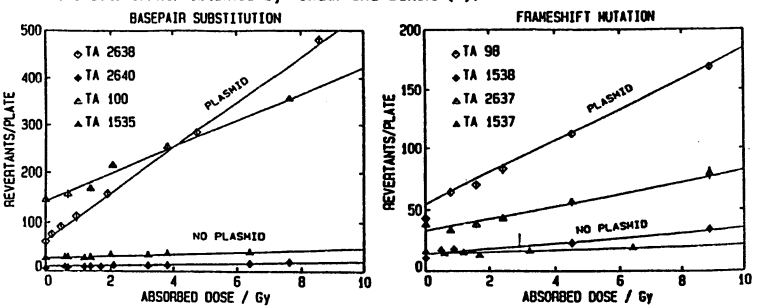

Fig.1 Number per plate of his$^+$-revertants of Salmonella tester strains versus the absorbed dose of ^{60}Co-γ radiation.
Panel A: Tester strains with base substitution hisG46: △ TA100, (plasmid pKM101); ▲ TA1535, (no plasmid). Tester strains with base substitution hisG428: ◇ TA2638, (plasmid pKM101); ◆ TA2640, (no plasmid). 2.7-5 10^8 bacteria were plated on each individual culture plate.
Panel B: Strains with histidine requiring frameshift mutations hisD3052: ◇ TA98, (plasmid pKM101); ◆ TA1538, (no plasmid). Frameshift mutation hisC3076: △ TA2637, (plasmid pKM101); ▲ TA1537, (no plasmid). 2-2.6·10^8 bacteria were plated on each individual culture plate. Standard errors are given for 12 plates which were irradiated per dose point.

The two panels of Fig. 2 permit a comparison of the reversion rate in the strains with plasmid and without plasmid. Two different parameters are utilized. The left panel gives the ratio of the number of revertants per plate obtained at 10 Gy to the number of spontaneous revertants per plate. The right panel gives the number of revertants per gray per plate, i.e. the slope of the dependences in Fig. 1. The values of similar parameters determined by Isildar and Bakale (1) are indicated by the horizontal lines superimposed on the bars.

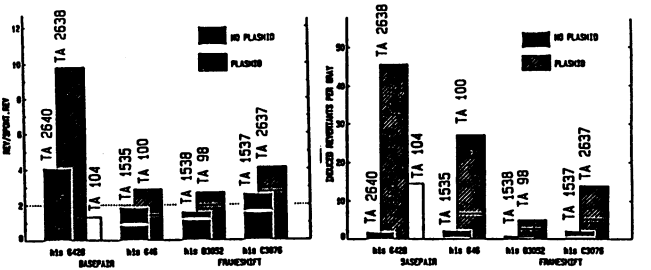

Fig.2 Ratio of revertants at 10 Gy to spontaneous revertants (left panel) and revertants per Gy (right panel) for different Salmonella tester strains with the plasmid pKM101 (hatched bars), and without it (full bars). Parameters obtained by Isildar and Bakale (1) are indicated by horizontal lines superimposed on the bars. Three strains with the base substitution mutation hisG428 are used, of which two (solid bar (TA2640), hatched bar (TA2638)) have the uvrB gene, responsible for the excision repair.

It must be noted that the values of Isildar and Bakale (1) do not refer to 10 Gy but to the highest values reached in their experiments which were obtained at substantially higher doses. The ratio of the reversion frequencies in the modified and in the conventional technique are, therefore, larger than the comparison in Fig. 2 would indicate. The data obtained with the present technique do not require a correction for lethality.

The data show the greatly enhanced frequency of spontaneous and radiaton induced revertants, which is caused in all strains by the introduction of plasmid pKM101, which contains the muc-gene and enhances the host's error prone SOS repair system.

The highest absolute and relative reversion rates are obtained in the three strains with the hisG428 mutation. One finds by far the highest spontaneous rate in TA104 which lacks the uvrB gene for excision repair; it is notable that the two other strains exhibit, in spite of the uvrB gene, absolute radiation sensitivities for reversion that are equal or even larger than that of TA 104.

The gain in sensitivity achieved by the modified technique is evident from Fig.1 where one recognices clear dose dependences for all strains. In the data from the earlier technique such dose dependences were established only for some of the strains. Even if the familiar two-fold rule which requires a doubling of spontaneous rates at arbitrary dose, is utilized one could show with the conventional technique the mutagenicity only in some of the strains.

b) VIABILITY

Inactivation studies were performed first with low dose rates of 0.16-0.8Gy/min, and it was then found that there were substantial differences for some of the strains to the data earlier obtained by Isildar and Bakale (1). Subsequent studies with a high dose rate of 25 Gy/min have essentially removed these differences. It has been found that there are substantial dose rate dependences for some of the strains, which happened to be those with the frameshift mutation. Fig. 3 gives two examples.

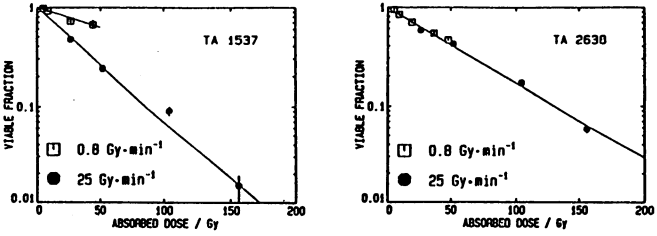

Fig.3 Fraction of viable bacteria after exposure to different doses of ^{60}Co-γ rays at different dose rates. Open symbols: low dose rate (0.16-0.8 Gy/min), closed symbols: high dose rate (25 Gy/min).
Panel A: Strain TA2638; (hisG428, plasmid pKM101).
Panel B: Strain TA1537; (hisC3076, no plasmid).

The inactivation data obtained at high and low dose rates are given in Tab. II which serves also as a synopsis for the reversion data in the different strains.

Genotype	his G428			his G46		his D3052		his C3076	
Plasmid pKM101	+	+	-	+	-	+	-	+	-
Strain	TA 2638	TA 104	TA 2640	TA 100	TA 1535	TA 98	TA 1538	TA 2637	TA 1537
Spontaneous revertants	55	510	6	145	27	31	14	45	14
Revertants at 100y	512	655	25	416	50	82	22	185	36
Slope (Gy^{-1})	45.7	14.5	1.9	27.1	2.3	5.1	0.8	14.0	2.2
Rev./Spont.	9.3	1.3	4.1	2.9	1.9	2.7	1.6	4.1	2.6
0.16-0.8Gy/min LD_{50}(Gy)	40	25	43	26	23	32	43	35	25
25Gy/min LD_{50}(Gy)	40	25	43	26	23	49	142	50	69
Ratio	1	1	1	1	1	1.53	3.3	1.43	2.76

A diagram to indicate the dose-rate dependences of inactivation is given in Fig. 4.

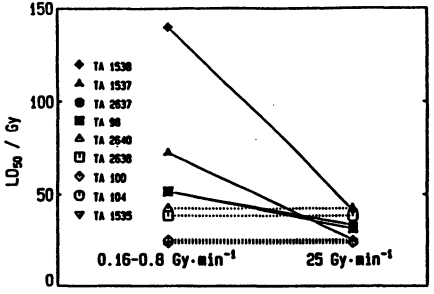

Fig.4 LD_{50} versus the dose rate for different Salmonella strains with base mutation (broken lines, open symbols) and frameshift mutations (solid lines, closed symbols).

There is no apparent correlation of the dose-rate effect with the presence or absence of the plasmid. The difference in the results is striking and suggests the need for more detailed studies of the dose-rate effect and the shape of the inactivation curves.

ACKNOWLEDGMENTS

These studies were supported by the Ministry for Environmental Protection and Reactor Safety, Federal Republic of Germany, under Contract St.Sch.956. The conclusions in this report are within the responsibility of the authors. We acknowledge the use of the γ-irradiation facilities of the GSF, and the generous help provided by Dr.W.Göggelmann (provision of laboratory facilities) and O.Balk (radiation exposures and dosimetry). This study has greatly profited from the dedicated work of Mrs.R.Enßer and Mrs.P.Wolf.

REFERENCES

1) M. Isildar, and G. Bakale (1984), Radiat.Res. 100, 396-411.
2) H. Roos, W.-H. Thomas, and A.M. Kellerer (1985), Radiat. Res.104, 102-108.
3) D.M. Maron, and B.N. Ames (1983), Mutat. Res. 113, 173-215.