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MUTATION INDUCTION BY CROSS-LINKS IN DNA OF *DEINOCOCCUS RADIODURANS*

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Reversion of adenine auxotrophic strains of *D. radiodurans* by cross-links in DNA was studied. Mutation was induced in a wild-type but not in a mitomycin C sensitive mutant after irradiation with near UV (∿365nm) in the presence of 4,5',8-trimethylpsoralen (TMP). To get information on implication of cross-links in the observed mutation, cells containing monoadducts in DNA were exposed to near UV in the absence of TMP. The mutation frequency of the wild-type increased with increasing the cross-links in DNA. These results suggest that structural defects after removal of cross-links in DNA induce mutation in *D. radiodurans*.

Deinococcus radiodurans is well known as an extremely radiation-resistant bacterium. Lesions including double-strand scissions in DNA induced by ionizing radiation are efficiently repaired during postirradiation incubation (1,2). It has been calculated that approximately 200 double-strand scissions or 190 cross-links per single chromosomal DNA induced by gamma-rays or mitomycin C, respectively, are repaired without any loss of viability (3∿5). This similarity in number of repairable lesions in DNA suggests that common pathway, if not all, may be involved in the repair of double-strand scission and of cross-link. Therefore, it seems very important to study repair of cross-links in DNA for elucidating the efficient repair mechanism in this bacterium.

In this study, we examined whether mutation was induced by cross-links in DNA of *D. radiodurans* although it was reported that the cell had a system by which many DNA lesions were repaired in an error-free manner (6,7).

MATERIALS AND METHODS

Strains; Adenine auxotrophic strains of *D. radiodurans*, KD830 and KI696, isolated by treatment with nitrosoguanidine, were used in this study (8).

Media and chemicals; The cells were grown at 30°C in a liquid medium (TGA) containing 5g of Bacto-tryptone (Difco), 1g of glucose, and 50μg of adenine in 1000ml of distilled water (pH 7.0). Two kinds of solid medium

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were made by adding 20g agar (Difco) per 1ℓ of TGA or TG (excluding adenine from TGA) for measurements of survivors and revertants (Ade^+), respectively. Methyl methanesulfonate (MMS) and 4,5',8-trimethylpsoralen (TMP) were purchased from Tokyo Kasei and Aldrich Chemical Co., respectively.

Far UV (254nm)- or gamma-irradiation; After the cells growing exponentially were harvested by centrifugation and washed with M/15 phosphate buffer (PB, pH7.0), they were suspended in the buffer ($\sim 10^9$ cells/ml). The suspension was exposed to UV (germicidal lamp, maximum energy output at 254nm) at 15 $J/m^2/sec$, or irradiated with ^{60}Co γ-rays at a dose-rate of 11 krad/min.

Treatment with methyl methanesulfonate (MMS); The cells suspended in PB ($\sim 10^8$ cells/ml) were treated with various concentrations of MMS at 37°C for 30 min. After they were washed with PB to remove the drug, the cells were plated on TGA or TG solid medium.

Treatment with psoralen-plus-light;
(i) TMP plus near UV: After the cells suspended in PB ($\sim 10^9$ cells/ml) were mixed with TMP (2.5×10^{-7}M) and allowed to stand for 30 min at 0°C, the mixture was exposed to near UV at 15 $J/m^2/sec$. The wavelength of near UV emitted from UV transilluminater (Ultraviolet Products, Inc.) was 300 to 380nm (peak intensity at 365nm).
(ii) [TMP plus light($\sim$400nm)] plus near UV: The cell suspension ($\sim 10^9$ cells/ml) mixed with TMP (2.5×10^{-7}M) as mentioned above was exposed to light (390 to 410 nm, peak intensity at 400nm) at 1.8 $J/m^2/sec$. The light was emitted from Xe-lamp (Watanabe, MX-150) and passed through two glass-filters, L-39 and KL-40 (Toshiba) for the irradiation. After exposure to light ($\sim$400nm), the cells were extensively washed with PB and resuspended in PB at the same concentration before the exposure. A second exposure to near UV ($\sim$365nm) was performed using the same method as described above.

Measurement of cross-links in DNA; Cells grown in TGA-broth containing [^3H]-thymidine (5μCi/ml) were treated with psoralen-plus-light as mentioned above. The cells were lysed and DNA was extracted by the methods as already described (9). DNA samples were mixed with NaOH and allowed to stand for 10min at room temperature. After neutralization with HCl, they were loaded on a hydroxyapatite column and eluted as already described (5).

RESULTS AND DISCUSSION

Upon exposure to near UV ($\sim$365nm), psoralen binds covalently with DNA and cross-links are formed between pyrimidines located in the opposite strands of duplex DNA (10, 11). When a mitomycin C sensitive mutant, KI696 was irradiated with near UV ($\sim$365nm) in the presence of TMP, it was more sensitive to the treatment than a wild-type strain, KD830 (Fig. 1(a)). The reverse mutation was induced in the wild but not in KI696 by this irradiation (Fig. 1(b)). However, cells of KI696 were mutated to adenine prototroph by treatments such as far UV (254nm), gamma-rays, or MMS (Fig. 2). This result indicates that KI696 has a system for induced mutation as in the wild-type. It was reported in a previous paper that cross-links in DNA induced by mitomycin C were not removed in this mutant cell (8). Since the bases cross-

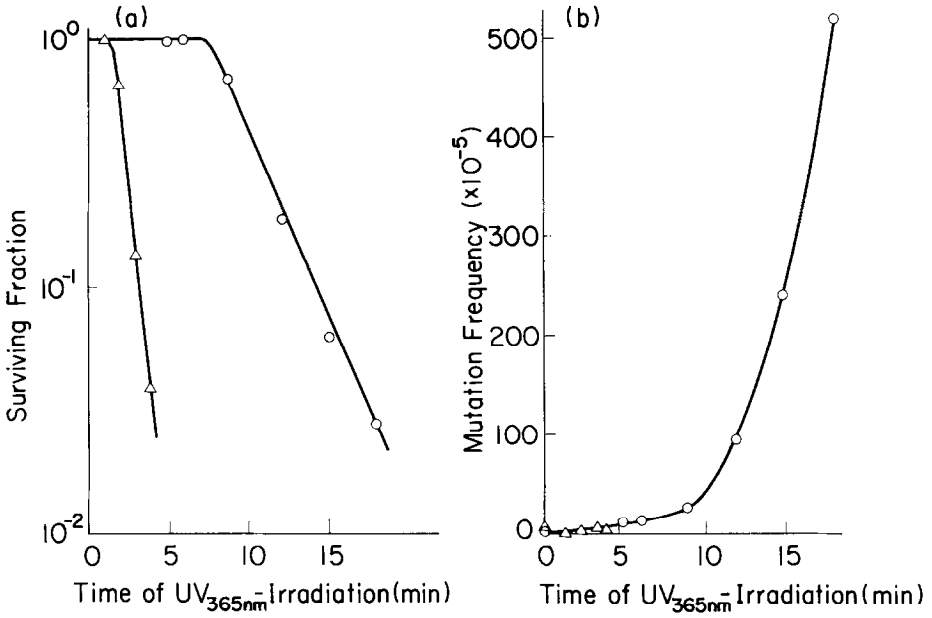

Fig. 1 Mutation induction in KD830 and KI696 after treatment with "TMP plus near UV(∿365nm)"; (a) surviving fraction and (b) mutation frequency. Symbols o and Δ represent the measurements for KD830 and KI696, respectively.

linked by psoralen-plus-light are thought to be different from those by mitomycin C (12), the amount of cross-links in DNA induced by "TMP plus near UV" was measured using the same procedures (5).

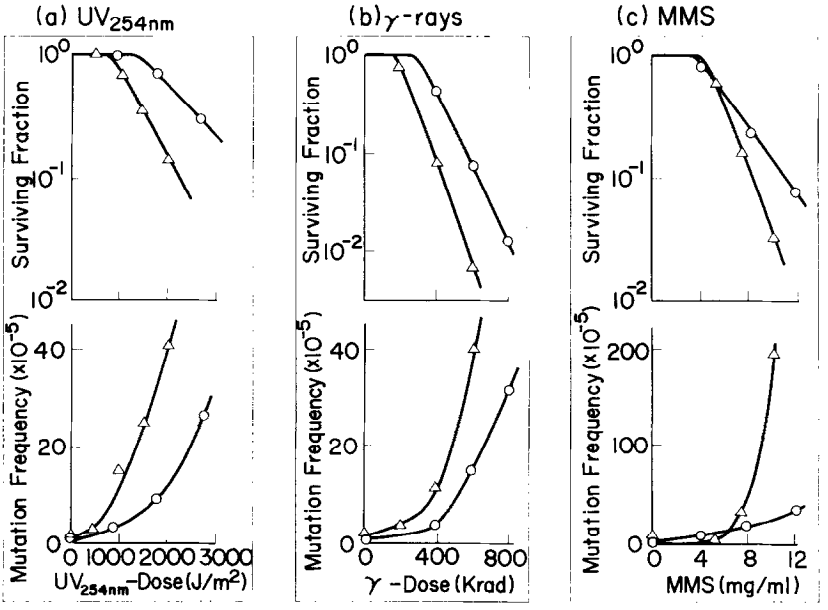

Fig. 2 Mutation induction in KD830 and KI696 after treatments as follows; (a) far UV (254nm), (b) gamma-rays, and (c) MMS. Symbols o and Δ represent the measurements for KD830 and KI696, respectively.

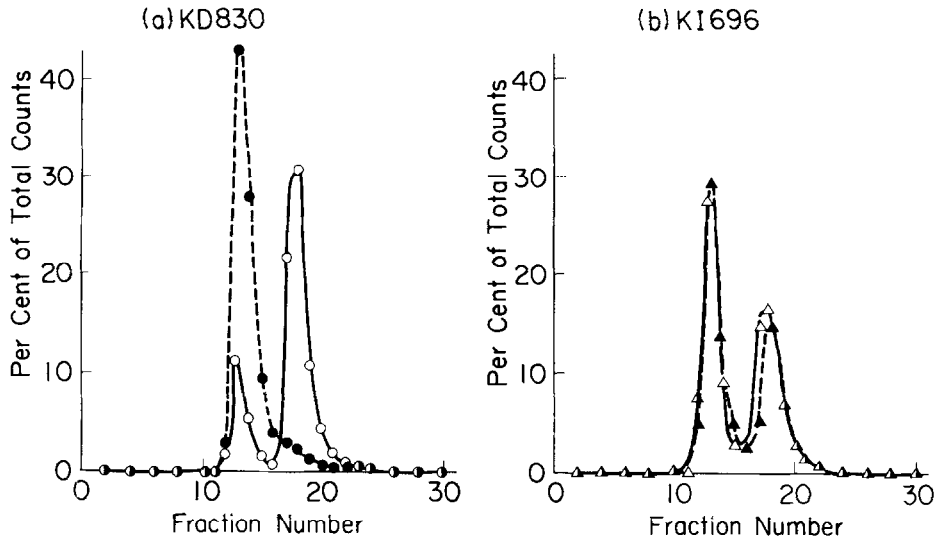

Fig. 3 Hydroxyapatite chromatography of DNA from the cells; (a) KD830 and (b) KI696. Cells of KD830 and KI696 were exposed to near UV(∿365nm) for 6 and 2 min, respectively, in the presence of TMP. Open and closed symbols represent the assays on DNA extracted from the cells immediately after the exposure or after postincubation of 6 hrs, respectively.

Relative amount of DNA containing at least one cross-link can be expressed by $\frac{RIds}{RIss + RIds}$, where RIss and RIds are radioactivities recovered from a hydroxyapatite column in fractions of single- or double-stranded DNA, respectively. As can be seen in Fig. 3 and Table 1, psoralen cross-links formed in the cells of KD830 were apparently removed, while they still remained in the mutant cells even after 6hr postincubation. Its high sensitivity to psoralen-plus-light suggests that cross-link in the mutant cell is a lethal lesion although the drug is known to make more monoadducts than the cross-links in duplex DNA (13, 14). On the other hand, it seems likely that cross-link in DNA may be a mutational lesion in the wild-type strain.

Table 1. Relative amount of DNA cross-links induced by "TMP + near UV(∿365nm)"

Strains	KD830				KI696			
Dose of near UV (min)	6				2			
Surviving fraction	1.0				0.69			
Postincubation time (hr)	0	2	4	6	0	2	4	6
RIds / (RIss + RIds)	0.78*	0.09	0.07	0.08*	0.49*	0.43	0.45	0.45*

*) These values were calculated from the chromatographic patterns in Fig. 3.

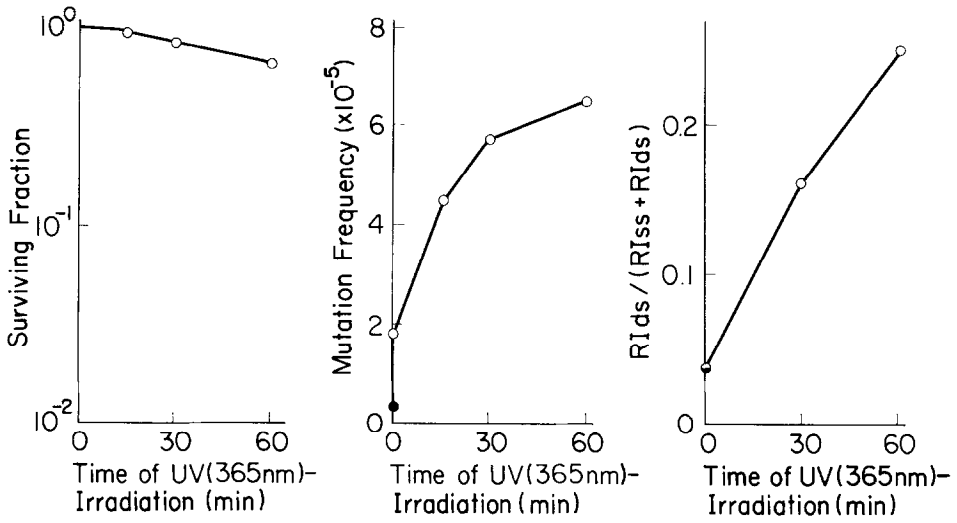

Fig. 4 Mutation induction in KD830 after irradiation in two steps; (a) surviving fraction, (b) mutation frequency, and (c) relative amount of DNA cross-links. The cells were irradiated with light (∿400nm) for 60 min in the presence of TMP. After washing out the drug, they were subsequently exposed to near UV (∿365nm). Values of RIds/(RIss + RIds) were calculated from the patterns of hydroxyapatite chromatography (not shown). Both values of mutation frequency and RIds/(RIss + RIds) were corrected for small contribution of TMP, which could not be removed by the washing before the second exposure. Closed symbols in (b) and (c) represent the measurements for unirradiated cells.

To get information about such prediction, the wild-type cells were irradiated in two steps, first they were exposed to light at ∿400nm with TMP and then the cells were irradiated at ∿365nm after washing out the drug. Recently, it has been reported that irradiation of DNA in the presence of TMP at wavelength between 380 and 400 nm produces efficiently monoadducts without any cross-links. Subsequent irradiation with near UV (∿365nm) results in cross-linking of almost half of the monoadducts (15). After irradiation at ∿400nm, 4% of DNA was eluted from a hydroxyapatite column into double-stranded DNA fraction. However, the same amount of radioactivity was recovered in this fraction even when [^{3}H]DNA from unirradiated cells was separated under the same conditions (Fig. 4(c)). Irradiation with light at ∿400nm for 60 min in the presence of TMP induced the mutation (Fig. 4(b)) but cell viability was not changed (data not shown). These results are consistent with the observation (15) that cross-linkable monoadducts are mainly produced by the irradiation at ∿400nm. These monoadducts are thought to induce mutation in the wild-type cells.

After removal of free TMP by extensive washing, the cells containing monoadducts were exposed to near UV (∿365nm). This second exposure decreased slightly the surviving fraction of the wild-type cells, while the mutation frequency increased with increasing the dose of second exposure (Fig. 4(a) and (b)). Relative amount of DNA cross-links in the cells produced by the second exposure was measured by the hydroxyapatite column chromatography. As shown in Fig. 4(c), relative amount of DNA containing at least one cross-link increased with increasing the dose of irradiation at ∿365nm. This is a good agreement with the increased mutation as stated above.

These observations suggest that structural defects after removal of cross-links in DNA induce mutation in *D. radiodurans*.

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REFERENCES

1. Dean, C. J., Feldschreiber, P., and Lett, J. T. (1966) Nature 209, 49-52.
2. Kitayama, S., and Matsuyama, A. (1968) Biochem. Biophys. Res. Commun. 33, 418-422.
3. Harsojo, Kitayama, S., and Matsuyama, A. (1981) J. Biochem. 90, 877-880.
4. Moseley, E. B. E., and Coplan, H. J. R. (1975) J. Bacteriol. 121, 422-428.
5. Kitayama, S. (1982) Biochim. Biophys. Acta. 697, 381-384.
6. Kerzman, G. (1975) Mutat. Res. 28, 9-14.
7. Tempest, P. R., and Moseley, B. E. B. (1982) Mutat. Res. 104, 275-280.
8. Kitayama, S. (1983) submitted for publication, J. Bacteriol.
9. Kitayama, S., and Matsuyama, A. (1975) Mutat. Res. 29, 327-332.
10. Musajo, L., Bordin, F., Caporable, G., Marciani, S., and Rigatti, G. (1967) Photochem. Photobiol. 6, 711-719.
11. Musajo, L., Bordin, F., and Bevilacqua, R. (1967) Photochem. Photobiol. 6, 927-931.
12. Weissbach, A., and Lisio, A. (1965) Biochem. 4, 196-200.
13. Cole, R. S. (1971) Biochim. Biophys. Acta. 217, 30-39.
14. Dall'acqua, F. (1977) Research in Photobiology, pp 245-255, Plenum, New York.
15. Chatterjee, P. K., and Cantor, C. R. (1978) Nucleic Acid Research 5, 3619-3633.