POSSIBILITY OF THE REPAIR OF DOUBLE-STRAND SCISSIONS IN *Micrococcus radiodurans* DNA CAUSED BY GAMMA-RAYS

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Received September 23, 1968

A substantial variance in the ability to repair radiation damage to DNA has been found among various types of bacteria, resulting in different radiosensitivities. For instance, increase in sedimentation rates of alkali-denatured DNA of irradiated cells during reincubation after exposure to X-rays, which is interpreted as the repair of single-strand scissions in DNA, has been observed for *E. coli* B/r, a resistant strain, but not for *E. coli* B_{s-1}, a sensitive strain(McGrath and Williams, 1966). However, most bacteria are assumed to be unable to repair double-strand scissions and Kaplan(1966) has presented an evidence for this view with *E. coli* K12 by sedimentation analysis on neutral sucrose gradients. On the other hand, Dean *et al*(1966) have suggested the capacity of *M. radiodurans*, which is a vegetative bacterium extremely resistant to ionizing and ultraviolet radiations, for repairing double-strand scissions in DNA. The purpose of the present paper is to examine this possibility by the sedimentation analysis of tritiated materials in *M. radiodurans* after γ-ray irradiation.

Experimental Methods. Culture conditions and growth medium for *M. radiodurans* were the same as those already described(Okazawa and Matsuyama, 1967). After labelling with ^{3}H-thymidine(20 μc/ml) in B-broth for 2-3 generations, the cells were harvested at the logarithmic phase of growth and washed with Tris buffer(Tris-HCl 10^{-2}M, $MgCl_2$ 10^{-3}M, pH 7.6). Cells resuspended in the same buffer were irradiated at room temperature with ^{60}Co γ-rays at a dose rate of about 9.3×10^{4} rads/hr. During postirradiation incubation, cells were harvested at intervals as indicated in Fig. 2, washed with Tris buffer and transformed into protoplasts by incubation for 25 minutes at 37°C with lysozyme(2 mg/ml). After chilling the

cells, a portion was lysed by pipeting them slowly into 0.1 ml of 1 % SDS which had been layered on the top of a 4.2 ml 5-20 % sucrose gradient at 5°C(adjusted to pH 7.6 with 10^{-2}M Tris-HCl) and centrifuged for 80 minutes at 30,000 r.p.m. using a RPS-40 roter in an Hitachi ultracentrifuge. The molecular weight of DNA was estimated on the basis of its observed distance of sedimentation (Burgi and Hershey, 1963). DNA isolated from M. radiodurans by the method of Marmur(1961), was used as a reference, the molecular weight of which had been calculated from the $S^{0}_{20,w}$ value.

Results and Discussion. M. radiodurans shows a sigmoidal survival curve for γ-rays under aerobic conditions(Fig. 1).

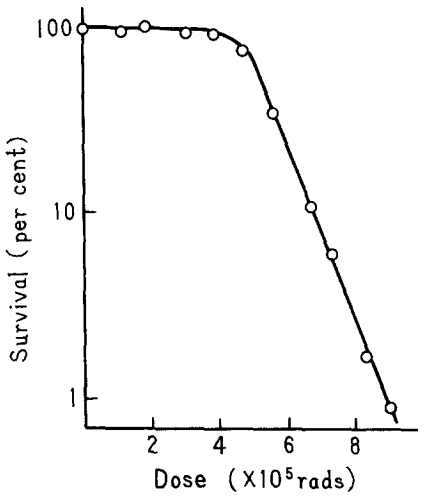

Fig. 1 The survival curve of M. radiodurans irradiated under aerobic condition.

Almost all cells were not inactivated with doses less than about 5-6x10^5 rads. Cells irradiated with 2.2x10^5 rads(≃100 % survival) and the unirradiated control were subjected to sedimentation analysis using neutral sucrose gradients. The results are illustrated in Fig. 2. The sedimentation rate of the main component of ^{3}H-labelled materials is decreased by irradiation(Fig. 2, A-B). The molecular weight was estimated as approximately 3.8x10^8 daltons for the unirradiated control and 1.8x10^7 daltons for the sample irradiated by 2.2x10^5 rads(one twenty-first of the unirradiated control). The sedimentation rate of the main component in the irradiated sample was increased by increasing the time of postirradiation

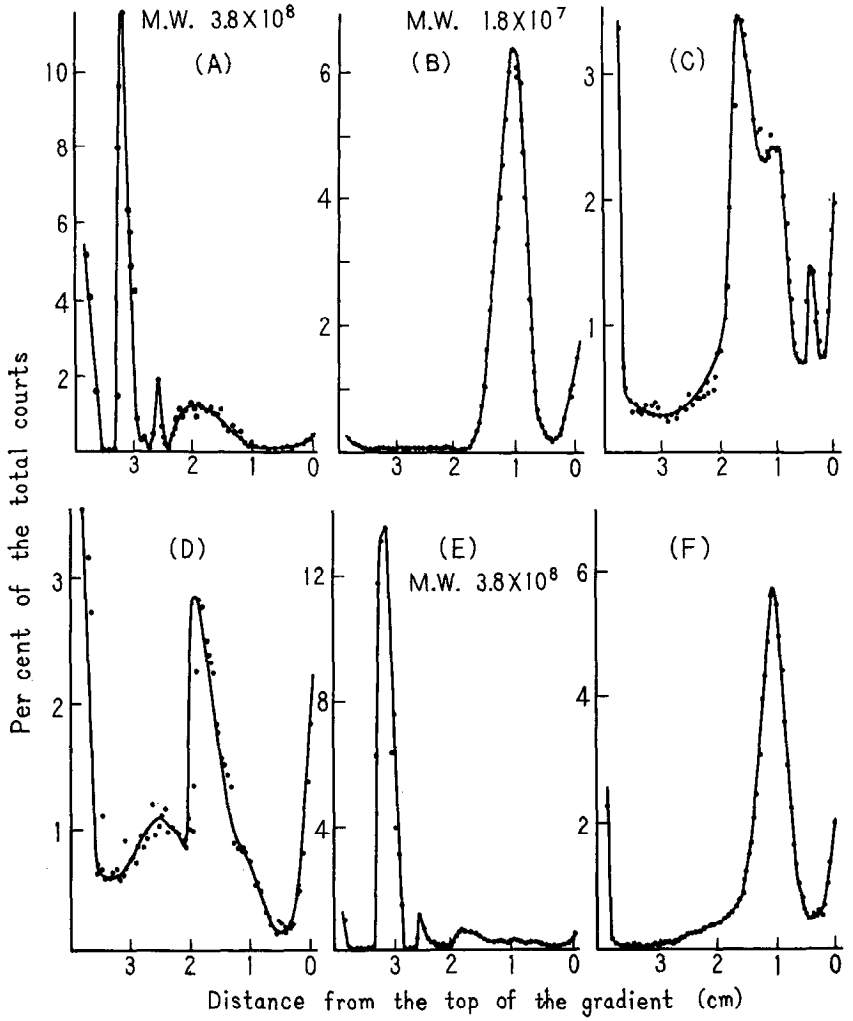

Fig. 2 Sedimentation patterns of tritiated materials in M. radiodurans, lysed and centrifuged on neutral sucrose gradients. (A) unirradiated control;(B) 2.2×10^5 rads, no incubation;(C) 2.2×10^5 rads and 60 minutes incubation;(D)2.2×10^5 rads and 120 minutes incubation;(E) 2.2×10^5 rads and 180 minutes incubation; (F)postincubation for 180 minutes in the presence of chloramphenicol at the concentration of 200 μg/ml.

incubation. After 180 minutes it was completely restored(Fig. 2, C-E). When the incubation was carried out in the presence of chloramphenicol(200 μg/ml), no detectable recovery of the sedimentation rate was observed(Fig. 2, F). The reduction of sedimentation rates on neutral sucrose gradients can be explained as the result of simultaneous breakages or of two lesions separated by very few nucleotides in both strands of DNA caused by radiation. Not to be excluded is the possibility of double-strand scissions occuring

at the sites of single-strand scissions in DNA during experimental treatment by enzymic action or hydrodynamic shear. As discussed by Dean et al(1966), such single-strand scissions caused by radiation would provide the weak point in DNA. Considering these two possible causes as mentioned above for the reduction of the sedimentation rate on neutral sucrose gradients, let us examine the results obtained in this research concerning the possibility of the repair of double-strand scissions in M. radiodurans DNA. The DNA content of a log-phase cell of M. radiodurans has been determined to be 3.5×10^{-14}g/cell(Okazawa and Matsuyama, 1967). Thus, with a dose of 2.2×10^{5} rads, the energy-loss events per cell are calculated to be 6×10^{3} on the basis that one energy-loss event corresponds to 60 eV(Rauth and Simpson, 1964). These energy-loss events may induce the DNA strand scissions in the range of $3.6\times10^{3}\sim 6\times10^{3}$ with the probability $p= 0.6\sim1.0$. If the initial ratio of probabilities of single- and double-strand scissions in the bacterial DNA on γ-irradiation is 37 as indicated by Munson et al(1967)(i.e. taking L_∞ as 0.40 keV/μ for ^{60}Co γ-rays and the probability of the single-strand scission remaining unrepaired u=1), the number of double-strand scissions in 3.5×10^{-14}g DNA as caused by 2.2×10^{5} rads is $95\sim160(=3.6\times10^{3}\sim 6\times10^{3}/38)$. Hence, it becomes likely that at least $2\sim3$ double-strand scissions$(95\sim160\times3.8\times10^{8}/3.5\times10^{-14}\times6\times10^{23})$ may arise in a chromatid of 3.8×10^{8} daltons with this dose. If it were assumed that single-strand scissions can be repaired while double-strand scissions are not repaired at all, incomplete restoration of the sedimentation rate of ^{3}H-labelled materials should be observed, thereby indicating smaller molecular weight(1/3∼1/4 or less) as compared with the unirradiated control. However, complete restoration was found after 180 minutes of postirradiation incubation, as shown in Fig. 2. This fact suggests the possibility of the repair of double-strand scission of DNA in M. radiodurans as caused by γ-rays. Another observation, that the colony survival at 2.2×10^{5} rads is almost 100 %, may strongly support this view. From the intrinsic viscosities of DNA extracted from irradiated cells, Dean et al(1966) estimated that at least five ruptures resulted from single breaks for every double break occuring in the M. radiodurans DNA upon X-ray irradiation in vivo.

Since the data obtained in this present study produces approximate values of 5.9 and 10 of this ratio for p=1 and p=0.6, respectively, the analysis of the data mentioned above lead to results not far

from the estimations of Dean et al.

Acknowledgements. The authors wish to express their thanks to Dr. Y. Okazawa for the gift of DNA isolated from M. radiodurans and also to Miss. N. Nakane and Mrs. Y. Miyazawa for their technical assistance. This work was supported by research grants from the Ministry of Education and from the Science and Technology Agency.

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