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Decreased Capacity for RNA Synthesis *in vitro* in a Membrane Fraction from *Micrococcus radiodurans* Irradiated by Ionizing Radiations

Sir:

In our recent studies on the mechanism for radiation resistance of *M. radiodurans*,¹⁻³⁾ the following results have been obtained: (1) remarkable radiation resistance of this vegetative bacterium is mainly due to the efficient capacity to repair DNA lesions including double-strand scissions, (2) the inhibition of protein and RNA synthesis during the postirradiation incubation resulted in the blocking of the repair of radiation-induced strand breaks in DNA, that is, DNA repair requires *de novo* protein and/or RNA synthesis, (3) the cellular sensitivity to ionizing radiations increased by the postirradiation treatment with either of actinomycin D, chloramphenicol and tetracycline, and (4) inhibition of protein and RNA synthesis in *M. radiodurans* cells increased as much as that of DNA synthesis with increasing radiation doses. Based on these results, it is assumed that inhibition of protein and RNA synthesis is of primary significance for the cell death in a radioreistant bacterium *M. radiodurans* caused by high doses of ionizing radiations.

In order to prove this view, the experiment using the cell-free system has been undertaken. The membrane fraction was prepared from unirradiated and irradiated cells of *M.*

radiodurans by lysing protoplasts with Triton X-100 according to the partial modification of Nisman's method.⁴⁾ Log-phase cells suspended in TM-buffer (Tris-HCl 10^{-2} M, MgCl₂ 10^{-3} M, pH 7.6) were irradiated by 4.5×10^4 rads and 8.5×10^5 rads of ^{60}Co gamma rays. They were then washed with TMS-buffer (Tris-HCl 10^{-1} M, MgCl₂ 1.5×10^{-2} M, sucrose 5×10^{-1} M, pH 7.6) two times and incubated with lysozyme (2 mg/ml) in the presence of EDTA (2×10^{-3} M) in TMS-buffer at 37°C for 30 min. After washing the protoplasts twice with cold TMS-buffer, Triton X-100 was added at a final concentration of 0.5% and the mixture was then chilled in an ice bath for 30 min. After lysing, the membrane fraction was collected by centrifugation at $14,000 \times g$ for 20 min, washed twice and resuspended in TMS-buffer. The reaction mixtures used in this experiment are shown in Table I. Incorporation of ^{14}C -ATP into the acid-insoluble fraction was regarded as a measure of RNA synthesis by the membrane fraction.

The results obtained in this experiment revealed that the activity of RNA synthesis in this bacterium is profoundly inhibited at a very high dose of 8.5×10^5 rads, while it is only slightly inhibited by 4.5×10^4 rads, as can be seen in Table I. This fact means that the inhibition of RNA synthesis would be due to the radiation damages in its synthetic

1) S. Kitayama and A. Matsuyama, *Biochem. Biophys. Res. Commun.*, **33**, 418 (1968).

2) A. Matsuyama and S. Kitayama, Abstracts of 4th International Congress of Radiation Research, Evian, France, pp. 140 (1970).

3) S. Kitayama and A. Matsuyama, *Agr. Biol. Chem.*, **34**, 1346 (1970).

4) B. Nisman, "Methods in Enzymology," Vol. XII, Nucleic Acids, Part B, ed. by L. Grossman and K. Moldave, Academic Press, New York, 1968, pp. 794.

TABLE I. EFFECT OF GAMMA IRRADIATION ON RNA SYNTHESIS *in vitro*

Enzyme fraction	Radiation dose before lysis ($\times 10^5$ rads)	Condition of incubation	cpm/0.5 mg Protein of the enzyme fraction
Membrane	—	No incubation	120
"	—	Complete	2861
"	—	Complete minus GTP, CTP and UTP	110
"	—	Complete plus DNase (5 μ g/ml)	100
Supernatant	—	Complete	65
Membrane	0.45	Complete	2165
Supernatant	"	"	78
Membrane	8.5	"	714
Supernatant	"	"	115

The complete reaction mixture contained the followings in μ moles/ml: Tris-HCl, 50; MgCl₂, 0.5; β -mercaptoethanol, 1.5; ATP, GTP, CTP and UTP, 0.4 each; ¹⁴C-ATP, 0.2 μ Ci. The reaction mixture contained 0.8~1.2 mg protein of the enzyme fraction. Incubation was carried out at 37°C for 20 min.

system, but not to secondary effects of radiation damages on permeability, active transport systems or energy generating systems. As previously reported,¹⁾ the colony survival was about 1 per cent for 8.5×10^5 rads and 100 per cent for 4.5×10^4 rads. Thus, the above view on the primary importance of the inhibition of the RNA synthesis for the cell death in *M. radiodurans* was supported by the present *in vitro* experiment. Since the inhibition of the DNA repair after 8.5×10^5 rads was much greater than that after 4.5×10^4 rads,³⁾ it appears that the RNA synthesis after irradiation may play an important role in the repair of DNA lesions caused by radiation in this radioresistant bacterium.

Evidence for the inhibition of protein synthesis by irradiation in a cell-free system prepared from *M. radiodurans* has not yet been obtained. However, in the preliminary trial the serious inhibition of this activity in cell-free extracts of *E. coli* was found after relatively high doses of gamma rays.

These findings mentioned above allow us

to maintain the view that the inhibition of protein and RNA synthesis may play a primarily important role in cell death in *M. radiodurans* caused by ionizing radiations, perhaps associating with the arrest of DNA-repair processes to rejoin double-strand scissions and inhibition of DNA replication, in other words, *de novo* protein and RNA synthesis is required during postirradiation incubation to repair double-strand scissions in DNA.

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