Biological Science

27B/2009G101

Effects of high-dose X-ray irradiation on stress-resistant microbes and biochemical substances

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Introduction

The main cause of DNA damage resulting from irradiation is the indirect action of water-derived OH radicals. Therefore, the radiation tolerance of dried microbes may improve without this indirect action of water-derived OH radicals. However, little is known about the radiation tolerance of dried microbes. In this study, the effects of irradiation on cells in a water-containing state (wet cells) and in a lyophilized state (dried cells), from which the water had been removed, were compared. Wet and dried cells of 3 types of bacteria, including *Escherichia coli*, the well-known radiation-tolerant bacterium, *Deinococcus radiodurans*, and the highly stress-resistant spore, *Bacillus subtilis* spore, were used for the irradiation tests. Their survivability was determined by the biological "energy currency", *i.e.*, adenosine 5'-triphosphate (ATP), and the most probable number (MPN).

Material and methods

In the irradiation experiment, synchrotron X-rays with a wavelength of 0.2 nm (6.2 keV) were used, exposure dose rate was 4.4 Gys^{-1} (500 Rs^{-1}), irradiation area was approximately 0.9 cm^2 (3 mm×30 mm), and absorbed doses were between 0 and 10,000 Gy. After irradiation, microbial samples were collected under sterile conditions, the re-suspended cell solutions were diluted, and an 8-tube MPN was performed. The number of surviving microbial cells was estimated from the dilution step for which microbial growth was observed. Furthermore, residual ATP concentration was measured with an ATP tester (AF-70; TOA DKK Electronics Ltd., Tokyo, Japan).

Results and discussion

Figure 1 shows survival rates after X-irradiation. In *E. coli* and *Deinococcus radiodurans*, survival rates were higher for dried cells than for wet cells. These results suggest that the damage resulting from water-derived reactive oxygen species was reduced. On the other hand, we observed that spores have no significant changes in the radiation tolerance of wet and dried cells. We think that since there is a small amount of free water in spores, there were almost no changes in intracellular water content as a result of drying. Our findings suggest that the drying of the cells restricted the formation and movement of water-derived reactive oxygen species, reduced cell damage, and under radiation exposure, increased the survival rate of microbes. Changes in intracellular ATP levels as a result of X-ray irradiation are shown in Fig. 2. In regard to X-ray irradiation, a decrease in ATP levels was observed only for wet cells of *Deinococcus radiodurans*, but no changes in ATP levels were found for dried cells of the 3 species. In addition, the ATP molecule showed no changes in ATP levels by irradiation in the wet or dry state (data not shown). Based on these findings, we think that ATP decreases in wet cells as a result of ATP consumption through metabolic functions.

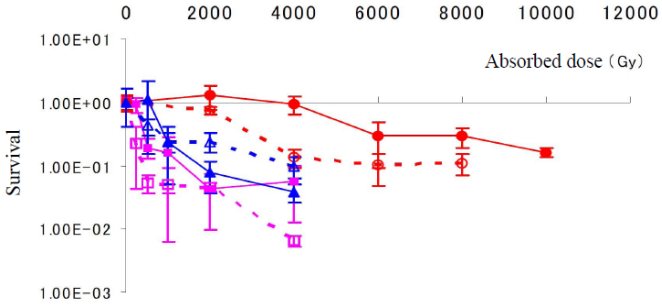

Figure 1. Survival of bacteria exposed to X ray.

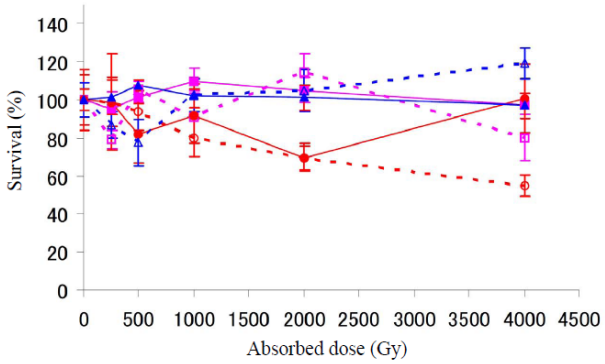

Figure 2. Survival of cellular ATP exposed to X ray

Wet (○) and dried (●) cells of *Deinococcus radiodurans*
Wet (△) and dried (▲) spores of *Bacillus subtilis*
Wet (□) and dried (■) cells of *Escherichia coli* K-12

References

[1] Y. Mashino, R. Nakai, T. Hasegawa, et al., Space Utilization Research 25: 188-191, 2009
[2] Y. Mashino, R. Nakai, K. Nakamura, et al., Space Utilization Research 26: 143-146, 2010

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