TEMPORARY INCREASE IN UV-RESISTANCE IN THE COURSE OF SPORE GERMINATION OF BACILLUS SUBTILIS

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The germination of bacterial spores has been defined as a physiological process characterized, e.g., by a loss of heat resistance, stainability, decrease in optical density and darkening under a phase contrast microscope(Campbell,1957). The present investigation revealed that a temporary increase in UV-resistance occurred in the course of germination of Bacillus subtilis spores.

Marburg strain of B.subtilis was grown on nutrient agar containing 0.1 mM $MnSO_4$ and clean spores were obtained by the method of Irie and Uchiyama(1964). After the activation of spores by heating at 70°C for 15 min., they were germinated at 37°C in M/15 phosphate buffer(pH 7.0) containing 5.6 mM L-alanine and 28 mM glucose, in which germination was completed within 20 min.

For the examination of UV-resistance, aliquots of the spore suspension were taken at intervals and promptly diluted(10^4-10^6 times) with sterile saline, transferred into Petri dishes, and subjected to UV-irradiation for various time length at room temperature (13-16°C). A Mazda 15 W germicidal lamp was used as the UV source at the distance of 30 cm. The irradiation density at the maximum wavelength

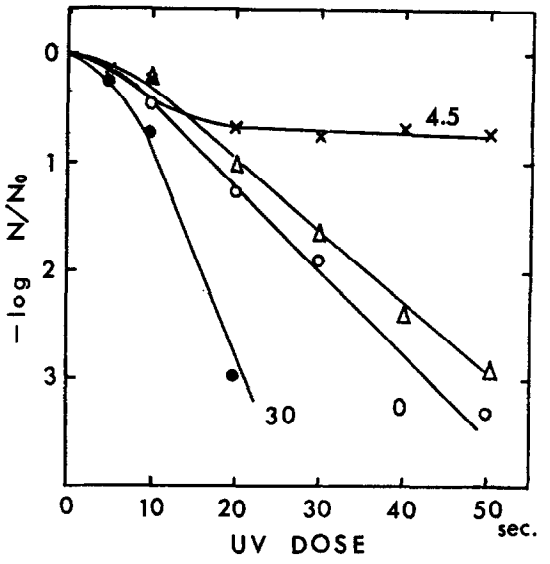

Fig.1. Inactivation of B.subtilis spores by UV light. Aliquots of the spore suspension were taken at 0,4.5 and 30 min. of germination and treated as described in the text. Survival curve obtained with non-heat-shocked spores is also illustrated (triangles). N: number of spores survived. No: number of viable spores before irradiation.

(2537) Å) was about 100 ergs/mm^2, sec. under the present experimental conditions (estimated by the courtesy of Dr. Y.Maruyama of Chiba University). After irradiation, nutrient agar containing $MnSO_4$ was poured into the dishes and they were incubated at 37°C for 1-2 days for the estimation of the number of surviving cells.

The dose response relationship shown in Fig.1 clearly indicates that at high doses actively germinating (4.5 min. incubation) spores are far more resistant to UV than non-germinated (0 min.) and germinated (30 min.) ones. It was also noteworthy that non-germinated and germinated spores were killed in a multiple-hit fashion (Morowitz, 1953), whereas in the case of spores incubated for 4.5 min., the curve consisted of two components with skewed portion at

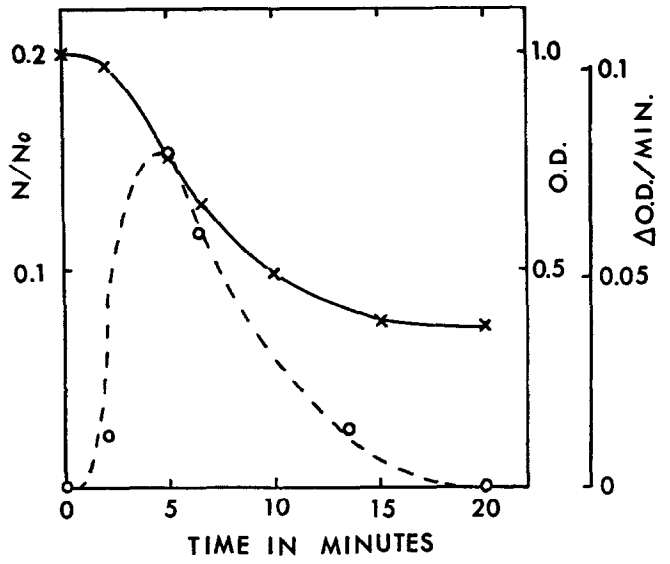

Fig.2. Changes in UV-resistance and optical density during germination of B.subtilis spores. Spores at various times of germination were irradiated for 50 sec. and survival fraction (N/No) estimated. Solid line with crosses: Optical density at 660 mμ(O.D.at time zero was taken as 1). Broken line: rate of decrease in optical density (calculated from time-O.D. curve). Open circles: survival fraction of spores.

higher doses. These observations suggest that considerably large fraction of spores temporarily becomes highly radioresistant in the course of germination. The time course of the change in radioresistance against 50 sec. UV irradiation was then followed during germination. As seen in Fig. 2, the surviving fraction of the spores was parallel to the rate of decrease in optical density of the suspension (open circles and broken line). Regarding the survival after 50 sec. irradiation to be virtually proportional to the total radioresistant fraction (x), we have

$$-dO.D./dt = kx$$

where k is a proportionality constant.

This relationship may be explained by assuming that germination of individual cell proceeds as following manner.

non-germinated → transient state → germinated

(refractile) (refractile, UV-resistant) (non-fractile)

Total fraction of spores at the transient state is expressed as x in the above equation. The mean life of the transient state is retained constant throughout the incubation period.

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