GERMINATION-INDUCED REPAIR OF SINGLE-STRAND BREAKS OF DNA IN IRRADIATED BACILLUS SUBTILIS SPORES

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Received August 5, 1969

SUMMARY. The single-strand breaks produced in DNA of B. subtilis spores by ionizing radiation were repaired during germination in the absence of normal DNA synthesis. This repair did not occur unless the initial stage of germination was induced.

It has been found by McGrath and Williams (1) and Kaplan (2) that single-strand breaks of DNA in irradiated Escherichia coli cells can be repaired during post-irradiation incubation.

In the present experiment, we found that this type of repair took place in bacterial spores during the germination process.

MATERIALS AND METHODS.

Bacterial strain: Bacillus subtilis (Marburg 168, thy$^-$ try$_2^-$) was used.

Preparation of spores and irradiation: Spores were prepared from vegetative cells by 24 hr incubation at 37°C in the sporulation medium of Schaeffer (3). Spores containing ^{3}H-labeled DNA were formed in the same medium supplemented with 10 μCi/ml ^{3}H-methyl thymidine (Radiochemical Centre, Amersham). After being harvested, the spores were treated with 500 μg/ml lysozyme and then 1 % sodium lauryl sulfate (SLS), and subsequently washed with distilled

water. Another portion of spores was treated with 10 % thioglycolic acid and 8M urea(4), The spores treated with such a reducing agent(reduced-spores) were still resistant to heating at 70°C for 30 min and possessed the same colony-forming ability as intact spores.

Irradiation was performed on intact or reduced spores in the wet packed state by a 6 Mev electron beam generated by a Varian linear accelerator at room temperature in open atmosphere.

Measurement of DNA synthesis during germination : Germination was carried out by shaking spores at 37°C in the medium of Demain(5), supplemented with 100 μg/ml L-tryptophan and 10 μg/ml thymine. The turbidity decrease of the culture was chased as a measure of germination. DNA synthesis was followed by incorporation of thymidine, where 10 μCi/ml ^{3}H-methyl thymidine was added to the culture at zero time of germenation. The incorporation was terminated by adding 10 % trichloroacetic acid and then the precipitate was collected on a Millipore filter for radioactivity count.

Measurement of single-strand breaks in DNA : Single-strand breaks of DNA in irradiated spores were estimated from zone sedimentation at pH 12, which was performed by essentially the same method as described by McGrath and Williams(1). Non-irradiated and irradiated intact spores were converted to spheroplasts without germination according to the method described previously (6). Another portion of irradiated intact spores was germinated for 70 min and then converted to spheroplasts by treatment with 500 μg/ml lysozyme in 25 % lactose, 0.01M EDTA and 0.01M Tris-HCl(pH 8.2), at 37°C for 10 min. Non-irradiated and irradiated reduced-spores were converted to spheroplasts by 500 μg/ml lysozyme with or without being germinated. These spheroplasts were ruptured by giving an osmotic shock and placed on a 0.1 ml layer of 0.5N

NaOH containing 1 % SLS which had been layered on top of the 5-20 % alkaline sucrose density gradient. Centrifugation was performed at 35,000 rev/min for 120 min at 20°C, and then contents were collected from top in fractions of 0.1 ml on a disk of filter paper. The disks were washed with cold 5 % trichloroacetic acid and then ethanol-ether, dried, and counted on radioactivity. The weight-average molecular weight of DNA was calculated from comparison of the sedimentation distance with those of T_4 and λ phage DNA according to the equation of Burgi and Hershey(7). Technical details are described elsewhere(8).

RESULTS AND DISCUSSION.

Thymidine incorporation into acid insoluble materials and the change of turbidity of the culture were chased during germination of intact spores following irradiation with various doses of electrons. The same observation was made on the reduced-spores. The results are shown in Fig. 1. In intact

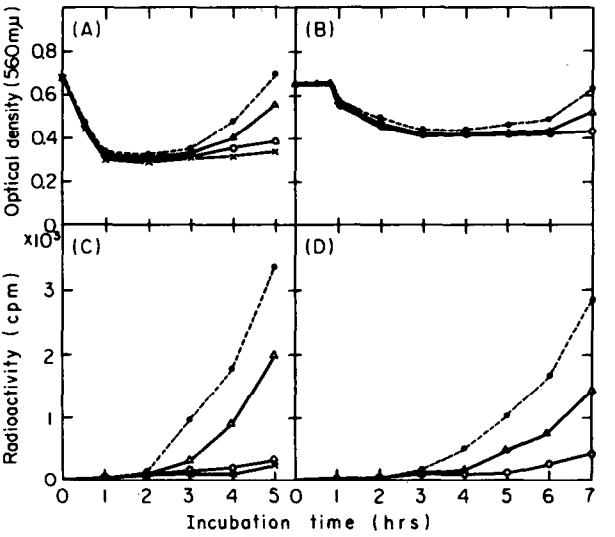

Fig. 1. Change of turbidity of suspension(A, B) and DNA synthesis(C, D) of B. subtilis spores during germination.
(A) (C) : intact spores.
(B) (D) : reduced-spores.
Non-irradiated control, ···●···; irradiated with 100 krad, —△— ; 220 krad, —○— ; 440 krad, —×—.

spores, the turbidity decrease seen at the initial stage of germination was not influenced by the electron irradiation. It seems that a typical pattern of the two-step increase of DNA synthesis in outgrowing spores(9) was maintained after irradiation. However, the rate of DNA synthesis was decreased by increasing radiation dose.

In reduced-spores, the turbidity decrease was initiated after the lag period of 40 min. The use of the reduced spores provides the advantage to observe the effect of germination with a prolonged time course and to facilitate the extraction of high molecular weight DNA at the initial stage of germination. Irradiation did not affect the turbidity decrease but lowered the rate of DNA synthesis, as similarly observed on intact spores.

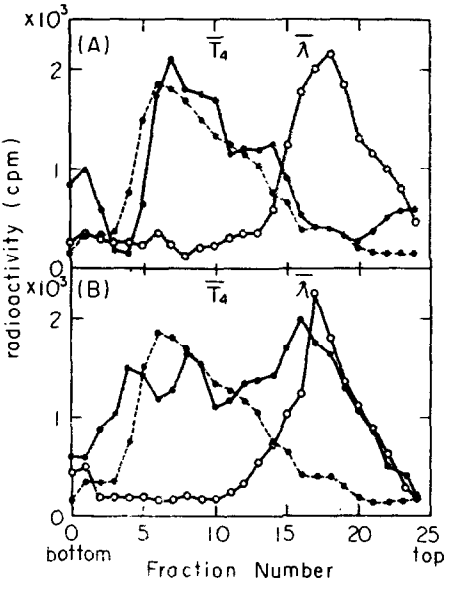

Fig. 2. Sedimentation patterns of DNA from irradiated spores(A) and irradiated reduced-spores(B) in the alkaline sucrose density gradient.
(A) ---●---, non-irradiated control; —○— , irradiated with 220 krad, no incubation ; —●— , irradiated with 220 krad, germinated for 70 min.
(B) ---●---, non-irradiated control; —○— , irradiated with 220 krad, germinated for 60 min ; —●—, irradiated with 220 krad, germinated for 150 min.
The symbols, $\overline{T_4}$ and $\overline{\lambda}$, indicate the peak position for T_4 and λ phage DNAs.

The sedimentation patterns in the pH 12 sucrose density gradients of ^{3}H-labeled DNA from non-irradiated and irradiated intact spores are shown in Fig. 2-A. The same experiments were made on DNA from reduced-spores (Fig. 2-B). When DNA was extracted from intact spores which were irradiated with 220 krad and allowed no germination, the sedimentation rate of the DNA was decreased. The post-irradiation germination for 70 min at 37°C restored the sedimentation rate to the level for DNA from non-irradiated spores, indicating that the single-strand breaks of DNA were repaired. The average molecular weight of single-strand DNA was 1.1×10^{8} daltons for non-irradiated spores, 0.9×10^{7} daltons for spores irradiated with 220 krad (5 % survival dose) and 1.0×10^{8} daltons for spores germinated for 70 min after 220 krad irradiation.

During the 70 min germination period after 220 krad irradiation, no ^{3}H-radioactivity was transferred from acid insoluble to acid soluble fraction but approx. 4 % of the total ^{3}H-radioactivity of DNA was released into incubation medium. This release was increased to 10 % by further 120 min incubation. On the contrary, in non-irradiated intact spores, approx. 1 % of the total ^{3}H-radioactivity of DNA was released into the incubation medium and another 1 % into acid soluble fraction during 70 min germination.

When reduced spores were irradiated with 220 krad, the DNA sedimentation pattern was found at the same position as that of intact spores irradiated with the same dose. The sedimentation pattern was not shifted during 40 min of lag period before the turbidity decrease began in germination medium. When the turbidity decrease was initiated, however, the DNA sedimentation pattern moved toward the direction of the sedimentation. The same effect was found when germination of the irradiated reduced-spores was induced by 0.1M L-alanine plus 0.06M phosphate buffer (pH 7.2) only. On the contrary,

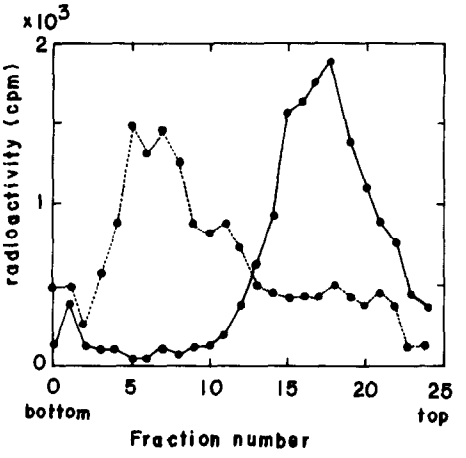

Fig. 3. Effect of L-alanine-induced germination on the DNA sedimentation pattern of irradiated reduced-spores in alkaline sucrose gradient. Symbols : —●— , irradiated with 220 krad, incubated in 0.06M phosphate buffer(pH 7.2) for 180 min ;---●---, irradiated with 220 krad, incubated in 0.1M L-alanine plus 0.06M phosphate buffer for 180 min.

incubation in 0.06 M phosphate buffer without L-alanine for 180 min at 37°C gave no effect on the DNA sedimentation pattern of irradiated reduced-spores (see Fig. 3).

The results shown above clearly demonstrate that the single-strand breaks of DNA in irradiated spores were repaired at the initial stage of germination before onset of the normal DNA replication.

We wish to thank Dr. H. Matsudaira for helpful discussions. Main part of this work was performed at National Cancer Center Research Institute.

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