Short Paper

Single Strand Breaks Induced in DNA of *Bacillus subtilis* Cells by Mild Heating

In the course of investigation on the heat resistance of spores of *Bacillus subtilis*, we found that when the spores were heated at 90°C for 10 min in distilled water auxotrophic mutants were frequently produced (unpublished). This finding suggests that the DNA molecules in the spores are modified by heating.

Several papers have been published concerning the relationship between the sensitivity to mild (50–52°C) heat or to ionizing radiation of several strains of *Escherichia coli*^{1–5} and *Salmonella typhimurium*^{6,7} and the occurrence of single strand scissions in DNA molecules as a consequence of heating. It is expected that such single strand scissions of DNA molecules caused by heating can also be found in *B. subtilis* in certain circumstances.

In this paper, we show our study concerning the degradation of single strand DNA by mild heating in the vegetative cells of *B. subtilis* and its relation to the heat sensitivity of the cells.

Logarithmic-phase cultures of *B. subtilis* 168 (thy⁻, trp⁻) (168 Tt) were inoculated to 10 ml of the modified Demain's medium⁸ supplemented with 20 µg/ml of tryptophan and 5 µCi of (methyl-¹⁴C) thymine. The cultures were grown at 37°C to an optical density (660 nm) of 0.6. The cells were then harvested, washed 3 times with M/15 phosphate buffer (pH 7.2), and resuspended in the same buffer. The suspensions were incubated at 50°C for various periods.

The cells were converted to protoplasts by the treatment with 500 µg/ml of lysozyme in 7% sucrose-EDTA solution. After centrifugation, the protoplasts were suspended in distilled water. By this treatment the protoplasts were osmotically disrupted and DNA was released. The DNA was assayed for single strand breaks by zone sedimentation through alkaline sucrose density gradients. For sedimentation, a 4.6 ml linear concentration gradient of 5–20% (w/v) alkaline sucrose (pH 12) was used. A 0.1 ml portion of 0.5 N NaOH containing 2% SDS and a 0.2 ml portion of ruptured vegetative protoplasts were layered on the top of the sucrose gradient by using a screw-pipette. The gradients were centrifuged at 30,000 rev/min for 90 min at 20°C in swinging-bucket rotors. After centrifugation, the contents were collected from bottom in fractions of 0.15 ml on disks of filter paper. The disks were dried and counted in toluene system in scintillation counter.

Figure 1 shows the sedimentation profiles for single strand DNA isolated from *B. subtilis* 168 Tt after 10, 20, and 30 min at 50°C. After 10 and 20 min incubation at 50°C about 10 and 5%, respectively, of the cells remained viable. The sedimentation distance of DNA was decreased in accordance with the decreasing of viability caused by heating.

Fig. 1. Sedimentation patterns in alkaline sucrose density gradients of DNA from vegetative cells of *B. subtilis*; —, not heated; - - -, heated at 50°C for 10 min; ·····, heated at 50°C for 20 min; — · —, heated at 50°C for 30 min.

The single strand breaks of DNA as a consequence of heating at 50°C suggests that the polynucleotide strand may be degraded by nucleases. The radioactivity of the top of the gradient was very little. Thus, the single strand scissions may not be related to exonucleases. Our observations are not readily reconciled with the DNA strand degradation by the direct action, such as hydrolysis or depurination, of elevated temperature. As described by SEDGWICK and BRIDGES⁵, these results are more consistent with attack by endonucleases released or activated by heating.

Aritsune UCHIDA, Yoshihiko SAKO,
and Hajime KADOTA

Laboratory of Microbiology, Department of
Fisheries, Faculty of Agriculture,
Kyoto University, Kyoto, Japan

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