Short Paper

Quantitative Analyses of the Spore Coat Protein in the Cell Extracts of *Bacillus subtilis**1

Because of the inertness of spore coat protein, it is very difficult to determine the quantity of it. In the course of the investigation on the biosynthesis of spore coat protein, we have made quantitative analyses of the spore coat protein in the cell extracts during sporulation of *Bacillus subtilis* by use of the polymerized antibody.

B. subtilis 3610 (ATCC 6051) was used as the wild-type sporulating strain. The strains 110NA(spoOA) and 11T(spoIV) were used as the asporogenic mutants. Cultures were grown in the Schaeffer liquid nutrient broth sporulation medium¹⁾ supplemented with 0.1 μ Ci/ml of ¹⁴C-labelled phenylalanine or serine. Cells were harvested at approximately one doubling time before and at the intervals of every 1 h after the end of exponential growth. The cells were disintegrated by sonication. The extracts were centrifuged in a refrigerated centrifuge at 10,000 rpm for 10 min. The supernatant fluids were again centrifuged at 18,000 rpm for 30 min and frozen at -20°C.

Antiserum to the spore coat protein was prepared and polymerized as described previously^{2,3)}.

Immunological binding assay for spore coat protein was done as follow: with the polymerized antibody concentration held constant (0.5 ml), various volumes of cell extracts (0.1 mg cell equivalent) were added to each assay tube and the total volume was adjusted to 1.0 ml by adding saline. The antigen-antibody mixture was incubated for 30 min at 37°C and then stood overnight at 5°C. Each sample was then centrifuged and washed three times with saline. The pellet fractions were assayed for labelled spore coat protein by calculating specific radioactivity of proteins.

Rifampicin (10 μ g/ml) was added to the culture at t_0 stage of sporulation.

The results are shown in Fig. 1. In the sporulating strain (wild-type), the spore coat protein appeared in the cell extracts at t_2 stage of sporulation. After that, the synthesis of the spore coat protein increased rapidly until t_4 stage. The concentration of the spore coat protein was about 40 μ g/mg cells at t_4 stage. There was no difference between the ¹⁴C-phenylalanine labelled cells and the ¹⁴C-serine labelled cells. After t_4 stage the concentration of the spore coat protein in the cell extracts decreased. This phenomenon may be due to the conversion of spore coat protein from the soluble form to the insoluble form. A similar result was reported with the spore coat protein of *B. cereus* T⁴⁾.

When rifampicin was added to the culture at t_0 stage, the appearance of the spore coat protein in the cell extracts was completely inhibited.

This is probably due to the non-specific absorption of proteins to the polymerized antibody³⁾.

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*1 *Bacillus subtilis* の孢子形成過程における細胞抽出液中の spore coat タンパク量の測定.

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Fig. 1. Biosynthesis of spore coat protein during sporulation of *B. subtilis* 3610 (ATCC 6051) and its asporogenic mutants.

○, 3610 (calculated with ¹⁴C-L-phenylalanine); ●, 3610 (calculated with ¹⁴C-L-serine); △, 3610 (rifampicin was added at t_0); ×, 110NA(spoOA); ◇, 11T(spoIV).

The 110NA(spoOA) mutant did not synthesize spore coat protein. On the other hand, 11T(spoIV) mutant synthesized a small quantity (about 8 μ g/mg cells) of the spore coat protein. In strain 11T the synthesis of the spore coat protein began as early as in the wild type strain, but after the stage t_2 a remarkable increase of the spore coat protein was not observed. This result indicates that some of the late stage mutants of *B. subtilis* were able to produce small quantities of the spore coat protein in a soluble form in the mother cells, and that they were not able to deposit the spore coat protein on the surface of spores^{2,3,5)}.

These results suggest that the biosynthesis of the spore coat protein begins at almost the same time of synthesis of alkaline phosphatase, a sporulation specific enzyme associated with stage II⁶⁾.

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