Bacillus subtilisのspore coatの可溶性タンパク質について

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Short Paper

Solubilized Protein from Spore Coat of *Bacillus subtilis**

It has been known that the major part of spore coat of bacterial spore is composed of protein which is highly hydrophobic and almost insoluble in dilute aqueous salt solution (1). Several attempts have been made to solubilize the spore coat by using reducing agents and denaturing agents (2, 3, 4, 5). However, the essential chemical character of the solubilized spore coat has not yet been made clear.

In the present study we tried to solubilize the spore coat protein of *B. subtilis* ATCC 6051 by the treatment of sodium dodecyl sulfate (SDS) and to determine molecular weight of the protein in a buffer solution added with SDS in order to obtain further information about the chemical structure of the bacterial spore coat.

Preparation of vegetative cell-free spores and isolation of spore coats from spores were made according to the methods previously reported (3, 7). The protein was extracted from a suspension in 0.1 M sodium borate buffer (pH 10.0) of the isolated spore coats (10 μg/m*l*) by the treatment at 50 C with 1% SDS and 0.1 M dithiothreitol. After being treated for 30 min the suspension was centrifuged at 15,000×g for 15 min. The supernatant fraction (SD-fraction) obtained was dialyzed against deionized water at 4 C for 48 hr and then lyophilized. By this treatment about 85% (dry weight basis) of the isolated spore coats was solubilized.

To examine electropholetic behavior of the protein in SD-fraction, the method of SDS polyacrylamide gel electrophoresis was used; the lyophilized SD-fraction (1mg) was dissolved in 1m*l* of 0.1% sodium borate buffer containing 0.2% SDS and 5% β-mercaptoethanol, and then subjected to the electrophoresis. The polyacrylamide gel (10%) containing 0.1% SDS and 0.05 M sodium borate buffer was used for the electrophoresis according to the method of Weber and Osborn (6). The electrophoretic mobilities of several kinds of authentic samples of protein (cytochrome C, chymotripsinogen A, ovalbumin, albumin and ovalbumin dimer), of which the molecular weights were known, were also examined for conparison's sake.

In the electrophoretic pattern obtained a straight line relationship was found between the electrophoretic mobility and the molecular weight of proteins. Based on these data molecular weight of the solubilized protein from *B. subtilis* spore coat was caluculated to be approximately 14,000. Aronson and Horn (4) also showed by using agarose column method and sucrose gradient method that protein in the SDS plus dithiothreitol soluble fraction from spore coat of *B. cereus* T had a molecular weight of 12,000 (4). Recently Uchida in our laboratory found by uses of immunochemical techniques and agarose column methods that molecular weight of a unit of the nascent spore coat protein present in sporulating cells of *B. subtilis* 6051 was approximately 15,000 (unpublished). Our data described above, together with the findings by Aronson and Horn (4) and those by Uchida, suggest that molecular weight of the unit (monomer) protein molecule in the spore coat of bacilli is 12,000 to 15,000 and that the unit protein molecules are associated to form a huge molecule in the spore coat of matured spore. Further studies on chemical structure of protein in the spore coat of *B. subtilis* are now in progress and will be published elsewhere.

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