Assembly and genetics of spore protective structures

H. Takamatsu and K. Watabe

Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka 573-0101 (Japan), Fax +81 72 866 3112, e-mail: watabe@pharm.setsunan.ac.jp

Abstract. The sporulation program in Bacillus subtilis ends in the formation of a highly resistant endospore that can withstand extremes of heat, mechanical disruption, ultraviolet irradiation, lytic enzymes and chemical attack. These properties are attributed mainly to the unique structure of spore coat and cortex, as well as to the physical state of the spore cytoplasm. The outermost layer of the spore, called the coat, has two morphologically distinct sublayers: an electron-dense outer coat and an electron-translucent inner coat. The coat is composed of more than 2 dozen proteins of varying size. Many coat genes and coat proteins have been isolated and characterized in detail, and studies of these have identified proteins with important roles in coat assembly, resistance and spore germination. We describe here characteristics of the coat proteins and propose a model for coat assembly based on recent work.

Key words. Bacillus subtilis; spore; coat protein; assembly; protective structure.

Introduction

The final step in the assembly of the Bacillus subtilis spore is the formation of the coat. This structure is essential for resistance and dormancy, at the very least by acting as a shield. One of the best-described protective functions of the coat involves acting as a barrier against lytic enzymes [1-7]. In addition to enabling the spore to survive periods of starvation, the coat also plays a role in the rapid response to the return of nutrient to the environment, known as germination [see review by Moir, this issue].

A typical ultrathin section electron micrograph of a dormant spore is shown in figure 1. The core, at the center of the cell, and the cortex, surrounding the core, are described in the reviews by Moir and Popham [this issue]. Surrounding these structures is the spore coat, consisting of a thick, electron-dense outer layer and a thinner, lamellar-like inner layer [2, 3], with between 25 and 35 proteins ranging in size from 8 to 65 kDa [4]. Spore resistance to harsh environmental conditions is due to both the complex spore structure and the unique physiological state within the core [1, 6]. Strikingly, the cortex and coat play two apparently opposing functions. First, they are protective structures essential to resistance and dormancy [1, 3, 5]. Second, they are critical to the prompt response of spores to molecules that trigger germination [8, 9].

In this chapter, we first review the morphology and composition of the spore-protective structures. Next, we briefly discuss each of the coat proteins and the regulatory factors, including sigma factors, which control coat protein gene (cot) expression. Finally, we propose a possible model for coat assembly based on recent results, and discuss a strategy for future research.

Coat morphology and composition

The spore coat has two morphologically distinct layers: a thick, highly electron dense outer coat and a less electron dense inner coat that appears to be further composed of a number of layers (fig. 1). The outer coat retains its electron-dense appearance even after sequential treatments with alkaline reagents and a mixture of proteases [10, 11]. The majority of the coat is composed of proteins [1-3, 12-14]. As the resistance properties of the spore suggest, solubilization of the coat proteins is quite difficult, even with harsh treatments such as 0.1 M NaOH, heat, sodium dodecyl sulfate, dithiothreitol, urea or combinations of these conditions [15]. Following these treatments only about 70% of coat proteins are solubilized.
with the rest remaining in an insoluble fraction. Although the biochemical basis of the large insoluble fraction is unknown, several lines of evidence indicate the presence of diverse species of protein cross-links that may connect the coat proteins. It has been suggested that both disulfide and o,o-dityrosine cross-links may be present in the coat assembly [2, 16]. Intriguingly, a spore-associated transglutaminase has been identified in \textit{B. subtilis} [17–19]. This activity directs creation of \(\varepsilon\)-(\(\gamma\)-glutamyl) lysine bonds between spore coat proteins. Protein cross-links could be a critical feature in spore resistance. Among the coat proteins that appear to be joined by cross-links are CotX and CotY (see below).

A typical example of the SDS-polyacrylamide gel electrophoresis (PAGE) profile of an extract of spore coat proteins from wild-type and mutant spores is shown in figure 2 [20]. Table 1 summarizes the major characteristics of the spore coat proteins of \textit{B. subtilis}. We have chosen to designate a protein as a coat protein only when its presence in the coat has been firmly established. We discuss our reasons for employing a relatively restrictive definition for coat proteins below. Here, we simply note that some putative coat proteins are omitted from table 1.

**Summary of the coat proteins**

Here, we summarize what is known about the \textit{cot} genes (and \textit{spo} genes that encode coat proteins) and their protein products. We also describe those ‘\(\gamma\)’ genes, identified as open reading frames of unknown function during the \textit{B. subtilis} genome project [21], which are now known to encode spore proteins with roles in coat formation.

**CotA, CotB, CotC and CotD**

CotA has a molecular mass of 65 kDa. The \textit{cotA} gene is identical to a previously identified gene called \textit{pig}, known to be responsible for sporulation-associated pigment production [22]. The expression of \textit{cotA}, \textit{cotB}, \textit{cotC} and \textit{cotD} genes (the latter three encoding proteins of 59, 12 and 11 kDa, respectively) is controlled by the sporulation-specific transcription factor \(\sigma^c\) and negatively regulated by the small accessory transcription factor GerE.