

Crystal Structure of Germination Protease from Spores of *Bacillus Megaterium*

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Beamline(s): X12C

Cells of *Bacillus* and *Clostridium* species produce spores when one or more nutrients are limiting for growth of these organisms. The spores DNA is saturated with a group of small, acid-soluble proteins (SASP) of the α/β -type. In spore germination SASP are degraded to amino acids and this degradation is initiated by a sequence specific protease called the germination protease (GPR). GPR is synthesized during sporulation in the developing spore as a zymogen. The GPR zymogen (termed P46) is a homotetramer with subunits of approximately 46 kDa, and is inactive both *in vivo* and *in vitro*. Later P46 undergoes intramolecular autoprocessing with removal of 7 to 16 N-terminal residues, generating an enzyme (termed P41), which is also a homotetramer and has full catalytic activity *in vitro*. Although P41 is catalytically active *in vitro*, it does not degrade α/β -type SASP in the dormant or developing spore, presumably because the conditions inside the core of the developing and dormant spore, particularly the dehydration, the low pH and the high levels of pyridine-2,6-dicarboxylic acid [dipicolinic acid (DPA)] and Ca^{2+} maintain P41 in an inactive state. However, early in spore germination the spore core rehydrates, its pH rises, and DPA and Ca^{2+} are excreted; these changes allow action by P41. This latter process frees up DNA for transcription, and also provides amino acids for protein synthesis during further development of the germinated spore (1).

The crystal structure of the P46 form of *B. megaterium* has been obtained by a combination of the multiwavelength anomalous dispersion technique (MAD) and twofold density averaging (2-3). The MAD diffraction data were collected utilizing selenomethionyl protein crystals at three wavelengths using the x12-C beamline at NSLS. The selenium atom positions were found by the automated structure determination of SOLVE. Using cautious model building and conservative refinement, the structure of P46 has been determined.

The final model of the P46 structure encompasses 320 residues (Figure 1). The asymmetric unit consists of two monomers related by a 2-fold NCS parallel. The crystallographic 2-fold axis produces the other two monomers of another asymmetric unit such that the four monomers form the functional tetrameric structure of this protease. The monomer adopts a folding pattern unlike that of any known protein. The unique P46 monomer fold can be divided into two domains: a core domain and a small cap domain (Fig. 1). The core domain contains a central mixed β -sheet. The cap domain consists of two α -helices. The two major α -helices are involved in forming a channel in the protein, and are stacked against the central β -sheet. The amino terminal ten residues of the protein extend away from the core domain into the solvent region near the surface of the molecule, and make few interactions with

Figure 1. Tetrameric structure of P46. Four monomers A, B, C, and D are shown in green, red, magenta and yellow respectively

the protein surface (4). The center of the tetramer has a unique channel structure (Figure 2).

References: (1) C. Nessi, *et al.*, "Studies of the structure and mechanisms of the protease that degrades Small, acid-soluble proteins during germination of *Bacillus* species", *J. Bacteriol.*, 180, 5077-5084, 1998. (2) P. Karthe, *et al.*, "Structural studies of a novel germination protease from spores of *Bacillus megaterium*", *J. Struct. Biol.*, 124, 19-24, 1999. (3) K. Ponnuraj, *et al.* "Crystallization and preliminary diffraction studies of a truncated form of a novel protease from spores of *Bacillus megaterium*", *Acta Cryst. D56*, 70-72, 2000. (4) K. Ponnuraj, *et al.* "Crystal Structure of a novel germination Protease from spores of *Bacillus megaterium*", *J. Mol. Biol.*, 300, 1-10, 2000.

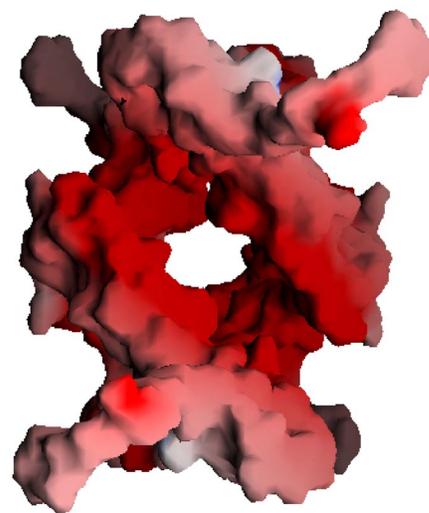


Figure 2. Molecular surface of the channel through the tetramer.