Supplementary Material

Crystallization and preliminary X-ray data analysis of the pXO1 plasmid partitioning factor TubZ from *Bacillus cereus*

Shota Hoshino, Takahisa Maki and Ikuko Hayashi

Protein expression and purification

The gene of Bc-TubZ was amplified by PCR from the genomic DNA of ATCC 10987 (Bacillus Genetic Stock Center) using primers of 5'-CCATGGCAGGCAATTTTTCAGAAATCGAAAGC-3' and 5'-GCGGCCGCTAGAATGATAATTTCTTCTTTGCTGGTTGTG-3' and cloned into *Nco*I/*Not*I digested pET21 (Novagen). All plasmids were transformed into *Escherichia coli* strain BL21(DE3) for protein expression. Cells were cultured in 1L of LB medium containing antibiotics (100 μg ml^{-1} ampicillin for Bc-TubZ and 50 μg ml^{-1} kanamycin for Bc-TubZΔ) with shaking at 37°C until A_{600} reached 1.0. IPTG was added to a final concentration of 0.1 mM and incubation continued for 3h. After induction, the cells were harvested, suspended in a lysis buffer containing 20 mM Tris pH8.0 and 0.15 M NaCl, and subjected to sonication for cell disruption. After the centrifugation at 17000 rpm for 20 min, recombinant Bc-TubZ protein was purified from the bacterial extract by 40% saturated ammonium sulfate precipitation. The protein precipitation was dissolved in 1 ml lysis buffer which was replaced on a PD10 column (GE Healthcare) with the lysis buffer with 1 mM DTT. The gene of Bc-TubZΔ was cloned into *Nde*I/*Not*I digested pET28a using primers of 5'-CATATGGAAAACCTGTATTTTCAGGGCAGCATGATGGCAGGCAATTTTTCAGAAATCG-3' and 5'-GCGGCCGCTAATTGGATTTCTTTGAATTAGCTTGCTC-3' with an additional TEV protease recognition sequence and expressed as a histidine-tag fusion protein (Blommel & Fox, 2007). The cell extract was loaded onto the HisTrap HP column (GE Healthcare) and the fusion protein was purified according to the manufacture's instruction. The protein fraction was dialyzed against a lysis buffer with 1 mM DTT and the tag was cleaved by addition of the histidine-tagged TEV protease for

16h at room temperature. After cleavage of the tag, the protein was loaded onto the ResourceQ column (GE Healthcare) pre-equilibrated with 20 mM Tris pH8.0, 5 mM β-mercaptoethanol. Proteins were eluted with a linear salt gradient (0-1M NaCl). Bc-TubZΔ fractions were pooled and concentrated to 0.5 mM in the buffer containing 10 mM Tris pH 8.0, 0.5 M NaCl and 1 mM DTT for crystallization.

Reference

Blommel, P. G. & Fox, B. G. (2007) *Protein Expr. Purif*. **55**, 53-68.