Structural Studies of DnaD Protein N-Terminal Domain from *Geobacillus kaustophilus HTA426* by X-ray Crystallography

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DNA replication is the most fundamental function in all biology. It is divided in three main stages known as initiation, elongation, and termination. Although DnaI is the gram-positive functional homologue of the *Escherichia coli* helicase-loader DnaC , both DnaD and DnaB have no homologues in gram-negative bacteria. They are essential for viability and required for both DnaA and PriA-mediated initiation of DNA replication. The molecular events that underpin the priming mechanism are unclear at present, but the data thus far indicate that DnaD interacts with DnaA, PriA, and DnaB , whereas DnaI interacts with the DnaC helicase . DnaD is believed to act early in the cascade setting the stage for helicase recruitment. DnaD and DnaB have global DNA-remodeling activities. DnaD forms scaffolds and converts supercoiled DNA to an open circular form, whereas DnaB compacts laterally supercoiled and linear DNA . The biological significance of these activities is not clear at present, but we have proposed a functional model with DnaD and DnaB being the link between global or local nucleoid remodeling and the initiation of DNA replication. Atomic force microscopy (AFM) revealed that DnaD converts all of the writhe of supercoiled plasmids into twist and suggested that this DNA remodeling might be accompanied by significant untwisting of the duplex . Untwisting of the DNA may compensate for the considerable force required to open up supercoiled plasmids without nicking. Once opened up, the plasmid is held firmly in the perimeter of a circular protein scaffold made up of DnaD molecules. Here we have get a crystal from the DnaD N-ter domain ($DnaD_N$). The X-ray diffraction data of $DnaD_N$ crystals were collected at BL12B2 Taiwan beamline at Spring-8, Japan.

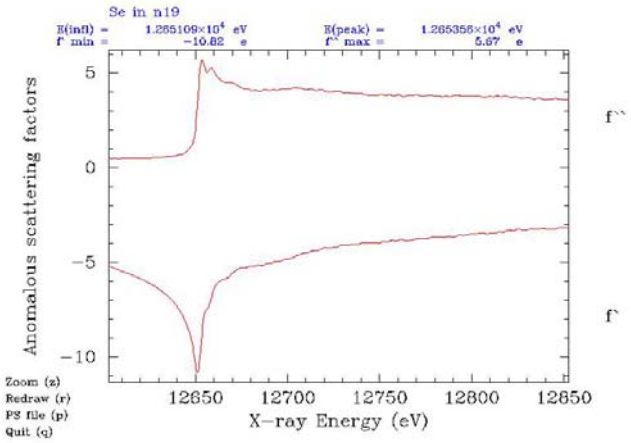

Figure 3. Anomalous scattering factors of Se-Met in GkDnaDn protein crystal.

The diffraction data were analyzed using HKL2000, and intensities were scaled with SCALEPACK. The three wavelengths (0.979401Å, 0.964263Å, and 0.979515Å) were chosen for the data collection of Se- $DnaD_N$ crystal. Here, the crystallization and preliminary analysis of the 18 kDa $DnaD_N$ protein, are reported. $DnaD_N$ has been crystallized at 277 K using $(NH_4)_2SO_4$ as precipitant. These crystals belong to the space group F222, with unit-cell parameters a = 116.77 b = 125.11, c = 157.643 A. A 98.5% complete native data set from a frozen crystal has been collected to 2.3 A resolution at 100 K. The presence of four subunit of $DnaD_N$ per asymmetric unit gives a crystal volume per protein weight (VM) of 2.45 and a solvent content of 49.78 %.