MS7-P15 Studies of the conformational changes on the ribosomal GTPase EFL1 using SAXS

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Ribosome biogenesis is closely linked to the cell growth and proliferation. Dysregulation of this process causes several diseases collectively known as ribosomopathies. One of them is the Shwachman-Diamond Syndrome, and the SBDS protein mutated in this disease participates with EFL1 in the cytoplasmic maturation of the 60S subunit. Recently, we have shown that the interaction of EFL1 with SBDS resulted in a decrease of the Michaelis-Menten constant (K_M) for GTP and thus SBDS acts as a GEF for EFL1 (1). Subsequent studies demonstrated that SBDS greatly debilitates the interaction of EFL1 with GDP without altering that for GTP. The interaction of EFL1 alone or in complex with SBDS to guanine nucleotides is followed by a conformational rearrangement. Understanding the molecular strategy used by SBDS to disrupt the binding of EFL1 for GDP and the associated conformational changes will be key to understand their mode of action and alterations occurring in the disease. The structure of the GTPase EFL1 is not known and its crystallization has been unsuccessful at least in our hands. In this study, we aim to show the conformational changes resulting from the interactions between EFL1 and its binding partners, the SBDS protein and the guanine nucleotides using SAXS technique (2,3). SAXS will provide structural information of the proteins and their conformational changes (4). For the SAXS data analysis we have built models of EFL1 using by EF-2 as homology template and of SBDS using the crystal structures of the archaea orthologues.

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Keywords: Elongation factor-like 1, Shwachman-Diamond Syndrome, BIOSAXS

MS7-P16 The structural basis of asymmetry in DNA recognition and catalysis:binding and cleavage by the I-SmaMI meganulease

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LAGLIDAG homing endonucleases (LHEs) are highly specific DNA cleaving enzymes, also termed 'meganucleases', that are used for genome engineering. These proteins are found both as homo-dimers and as pseudo-symmetric single-chain monomers. Like many other enzymes that act on DNA targets, meganucleases often display highly asymmetric DNA recognition properties, with the overall binding affinity and cleavage activity dominated by interactions between one protein domain and a corresponding DNA half-site. While the importance of asymmetric target recognition, binding and cleavage by meganucleases is important both with respect to their natural function and to their engineering in the lab, the structural basis for that behavior has not been well understood. Here we describe a systematic biochemical and structural analysis for a single-chain meganuclease, I-SmaMI, in which we determined the structure of the wild-type enzyme in the absence of bound DNA, in a complex with uncleaved DNA, and in a complex with cleaved DNA product. Structural comparisons of these structures, combined with binding and cleavage analysis of the wild-type and mutated variants of both the enzyme and substrate, demonstrated the asymmetric 'dominance' by one protein domain over the other during DNA recognition, binding and cleavage of the pseudo-symmetric target site. Information gained from the current study of the I-SmaMI is expected to contribute significantly toward the understanding and reengineering of other meganucleases.

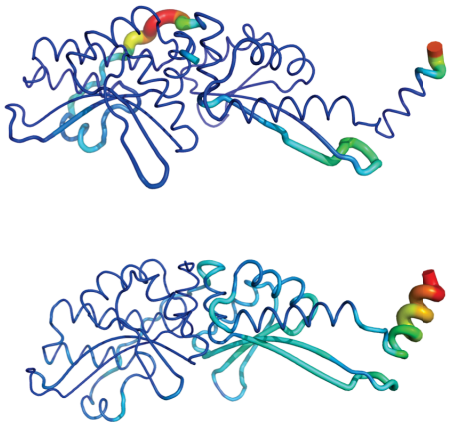

Figure 1. Putty cartoon representations of Apo I-SmaMI model. Top: colored according to B-factor of individual Cα atoms. Bottom: colored according to normalized RMSD between the Cα atoms of unbound and un-cleaved DNA bound I-SmaMI structures.

Keywords: LAGLIDAG, himing endonuclease, meganuclease