Structural and biochemical characterization of the mitomycin C repair exonuclease MrfB

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Nucleic Acids Research, Volume 52, Issue 11, 24 June 2024, Pages 6347–6359

Presenter : Chia-Yi Lin **Date/Time** : 2024/09/19 15:10-16:00

Commentator : Chyuan-Chuan Wu Ph.D. **Location** : Room 601, Med College Building

Background

DNA repair is critical for maintaining genome stability in all organisms, particularly in response to chemical agents like Mitomycin C (MMC), which induces DNA damage through monoadduct formation and crosslinking. Traditionally, MMC-induced DNA damage is repaired by the nucleotide excision repair (NER) pathway. In *Bacillus subtilis*, the MrfAB (Mitomycin repair factor AB) pathway functions independently of the NER system to repair MMC-induced damage, with MrfA acting as a 3' to 5' helicase and MrfB as a DEDDh exonuclease. Recently, the biochemical activity and structure of MrfA have been characterized, but much less is known of MrfB.

Objective

To investigate the biochemically activity and the crystal structure of MrfB exonuclease core domain.

Results

First, the authors determined that MrfB is a 3' to 5' magnesium-dependent exonuclease. Next, they solved the structure of the MrfB core domain through X-ray crystallography. Unexpectedly, the structure revealed an inactive state in the absence of DNA, with a displaced catalytic residue, Asp172. To explore the importance of specific residues, site-directed mutagenesis was performed. As expected, mutations in the DEDDh residues significantly decreased exonuclease activity in both ssDNA and dsDNA substrates. The basic loop, responsible for substrate binding, retained normal function when individual residues were mutated, but the enzyme lost activity when all basic loop residues were mutated simultaneously. Leu113, which acts as a wedge to break terminal base pairing, exhibited reduced activity and a prolonged initial phase of degradation. Finally, in vivo experiments revealed that the L113A mutation, as well as simultaneous mutations in the basic loop, failed to complement the *ΔmrfB* phenotype under MMC treatment, indicating their critical role in MrfB's function in removing MMC-induced damage.

Conclusion

This study provides the first structural and biochemical characterization of MrfB, a key enzyme in the MMC repair pathway of *Bacillus subtilis*. Future studies will focus on understanding the detailed mechanism of the MrfAB system.