Theoretical study of the catalytic mechanism of a new class of endonuclease using a histidine to form a protein-DNA adduct

Hansel Gómez¹,², Modesto Orozco¹,²,³

¹Institute for Research in Biomedicine (IRB Barcelona), Baldri Reixac 10-12, 08028 Barcelona, Spain.
²Joint BSC-CRG-IRB Program in Computational Biology, Barcelona, Spain.
³Department of Biochemistry and Molecular Biology, Biology Faculty, University of Barcelona, Barcelona, Spain.

In the present work we focus on a new class of endonuclease/ligase (i.e. MobM). These enzymes are also called relaxases, as their relaxes the supercoiled DNA and initiates its mobilization to another bacterial host of the same or different species. This process of horizontal gene transfer is responsible for rapid bacterial evolution and adaptation as well as antibiotic resistance spread among pathogenic bacteria. Relaxases and other enzymes of the HUH endonuclease superfamily depend on a single metal ion for the activation of the scissile phosphate bond and on a catalytic residue (typically a tyrosine), which performs the nucleophilic attack to form a protein-5'-DNA complex.

We performed Quantum Mechanics / Molecular Mechanics (QM/MM) calculations to describe the catalytic mechanism followed by MobM. According to this study, H22 is the catalytic residue that nucleophilically attacks on the scissile phosphate to form the DNA-protein adduct through a S₈₂²-like mechanism. We also identified E129 as a general acid that protonates the leaving O₃¹ group to support the nucleolitic reaction (See Figure below). Moreover, the relevant roles of residues H22 and E129 are fully supported by mutagenesis studies.

This is the first description of an enzyme using a metal/histidine catalytic machinery for DNA cleavage and ligation. Accordingly, our study provides with relevant information that could be used in the rational design of specific inhibitors of MobM or related enzymes that are therapeutic targets in pathogenic bacteria.

Keywords: Nuclease, Catalytic Mechanism, Quantum Mechanics / Molecular Mechanics (QM/MM).