Investigation of the Phosphodiester Cleavage Mechanism Catalyzed by the Metalloenzyme Endonuclease V Using a Holistic Computational Approach

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Although our genetic information needs to be error-free to maintain the proper functioning of cells, DNA is prone to environmental and endogenous damage. If left unrepaired, these lesions can lead to serious outcomes like mutations and cancer. To maintain the genomic integrity, cells possess different repair enzymes that remove the damage by cleaving the phosphodiester bond in nucleic acids. Endonuclease V (EndoV) is one such enzyme that removes deaminated lesions such as deoxyinosine/ inosine arising from spontaneous hydrolysis or nitrosative reactions of DNA or RNA. Unrepaired deaminated lesions have the tendency to mispair with canonical bases to introduce mutations during replication or transcription.

EndoV is a particularly interesting family of enzymes since, while the sequences are conserved across different domains of life, they have widely different functions. For instance, the central role of the bacterial enzyme is to remove deaminated DNA bases as part of DNA repair. In contrast, human EndoV (hEndoV) has a major role in RNA metabolism. However, no computational work has been done to date to explain the reasons behind the different roles performed by these very similar enzymes.

In the present work, we have used a multiscale approach, including molecular dynamics (MD) simulations (AMBER) and quantum mechanics-molecular mechanics (ONIOM(M06-2X/6-311+G(2df,p):AMBER) //ONIOM(B3LYP(D3BJ)/6-31G(d,p):AMBER)) calculations, to provide the first clues about enzyme function, including structural information about the DNA-–protein complexes, and the roles of metals and potentially important active site amino acid residues. As a result, this work provides fundamental knowledge regarding how endonucleases efficiently catalyze the phosphodiester bond cleavage in nucleic acids. These mechanistic details can pave way for designing powerful new drugs (chemotherapeutics) and expand their interdisciplinary applications required by bioindustries.