Using MD and ONIOM Calculations to Reveal the MutY Crosslinking Mechanism

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DNA is an important biological molecule that stores the majority of the genetic information of a cell. DNA is composed of four unique nucleobases, namely adenine (A), guanine (G), cytosine (C), and thymine (T). The A:T and G:C complementary base pairing ensures the integrity of the genetic code is maintained during DNA replication; however, DNA damage can disrupt the complementary pairing. 8-oxo-guanine (8oG) is a common form of DNA damage that is estimated to arise 1000 times per cell per day. 8oG can form a Hoogsteen base pair with adenine, resulting in an 8oG:A mispair, which can further lead to a G:C to T:A transverse mutation after a second round of replication. To prevent this outcome, cells utilize adenine DNA glycosylase (MutY) to detect 8oG:A mispairs and remove the adenine. The result is a 8oG:C damaged pair, which can be repaired by MutM to return to the canonical G:C pair.

There is an urgent need for a better understanding of the MutY mechanism of action, as there is a correlation between variations in MUTYH (the human homologue of MutY) and colorectal polyposis in a disorder called MUTYH-associated polyposis (MAP). MAP causing MUTYH mutants have been shown to have reduced rates of adenine removal, and some mutants have mutated amino acid residues in the active site, which links MAP to the deglycosylation mechanism. Insight into the mechanism of MutY may enable us to determine how specific MUTYH variants impact the enzyme function and thus would help predict the severity of, and develop possible treatments for, MAP. Several mechanisms for MutY have been proposed in the literature; however, the two-step mechanism that is currently the most consistent with experimental results is conjectured from crystallographic and mutational data, and lacks the atomic level of detail that computational chemistry can provide.

In this project, the mechanism of action of MutY is investigated using molecular dynamics (MD) simulations and QM/MM (ONIOM) calculations. MD simulations were used to investigate the dynamics of the active site of the wild type enzyme as well as a computationally generated MAP mutant. These simulations considered both the reactant complex and the crosslinked intermediate, providing the first structural depiction of this complex. From these MD simulations, ONIOM models were developed to map the reaction mechanism and provide atomic level details of the deglycosylation pathway for both the wild type and mutant enzymes. Overall, this work provides the first computational support for a unique MutY mechanism that involves the formation of a DNA–protein crosslink and gives insights into how MAP-causing mutants can impact the deglycosylation reaction.