Effect of N140D and T197A mutations on DNA repair enzyme Thymine DNA Glycosylase

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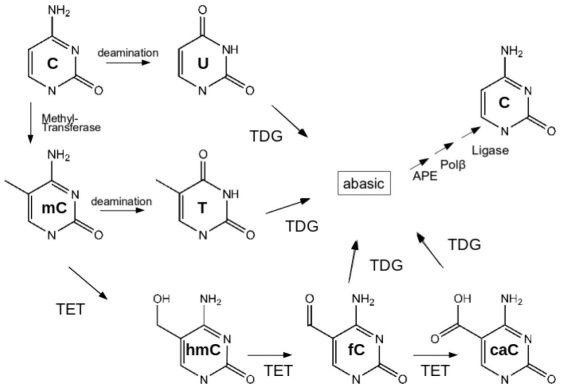
Thymine DNA Glycosylase (TDG) is an enzyme that participates in the DNA repair mechanism. Its primary role is to identify, and excise modified and mismatched bases, more specifically, Thymine, Uracyl or and oxidized form of Cytosine [1,2].



TDG's reaction mechanism involves the placement of a water molecule, that acts as a nucleophile, by two residues: N140 and T197 [3]. Mutating these residues inactivates or severely reduces enzyme activity. Interestingly, N140D mutated TDG is inactive against most substrates (except carboxyl cytosine), even though D140 could replace N140 in nucleophile placement [4].

Through atomistic MD simulations, we observed that a hydrogen bond network, involving N140 and T197, is crucial for the stability of the Protein-DNA complex formed during the excision mechanism. In the N140D mutation, the aspartate is unable to maintain most of the interactions of the network, and thus the complex becomes unstable, rendering TDG inactive. Protonation of D140 slightly alleviates this issue, acting as a surrogate of the amino group of N140, but the interactions formed are weaker than the wild-type enzyme. For T197A, some interactions of the network are kept, but the complex is not as stabilized as in wild-type, reducing TDG's activity drastically.

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[4] H. Hashimoto, X. Zhang, X. Cheng, *DNA Repair (Amst)* **2013**, *12(7)*, 535-540.)