Effect of N140D and T197A Mutations on DNA Repair Enzyme Thymine DNA Glycosylase

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Background

Thymine DNA Glycosylase (TDG) is an enzyme that participates in the DNA repair mechanism base excision repair (BER) by identifying and removing mismatched or modified bases. It is capable of excising thymine and oxidized forms of cysteine (figure 1) [1,2].

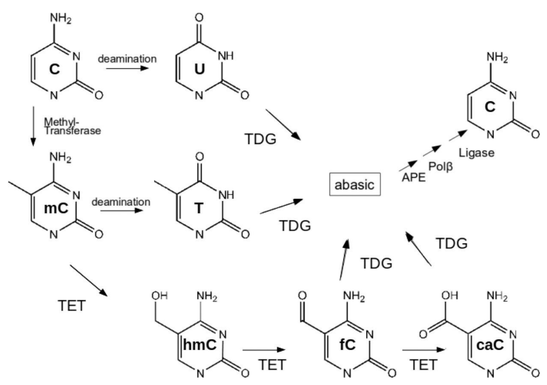

Figure 1. Possible pathways for cytosine modification and their ability to be recognized by TDG, where caC is carboxyl cytosine; fC is formyl cytosine; T is thymine and mC is methyl cytosine.

The base excision mechanism involves “flipping out” the substrate base into the active site of TDG, and the positioning of a water molecule (that acts as nucleophile) through interactions with N140 and T197. R275 fills the “gap” left by the flipped base, stabilizing the Protein-DNA complex (figure 2) [3].

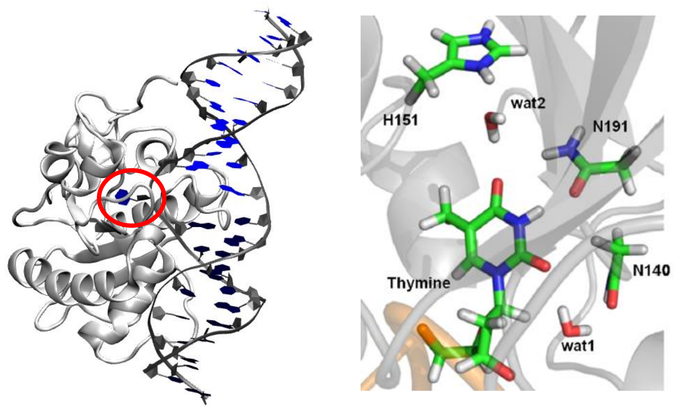

Figure 2. Left: Three-dimensional model of TDG interacting with DNA with the substrate base in its flipped conformation (based on PDB structure 6U17 [4]). Right: Schematic representation of the active site of TDG [3].

The mutation T197A reduces the activity of TDG by 30-fold. Nevertheless, TDG with the mutation N140D is inactive with most substrates and slightly active with carboxyl cytosine, even though an aspartate residue can position the nucleophilic water molecule [5]. This leads to the question: Which interactions are changed by the N140D mutation that render the protein inactive?

Methods

- Atomistic MD simulations (three replicas, 1 µs each) of:
  - ❖ Wild-type (WT) TDG with substrates: Thymine, carboxyl cytosine*
  - ❖ Mutated N140D (protonated and unprotonated) TDG with substrates: Thymine, carboxyl cytosine.
  - ❖ Mutated T197A TDG with substrate: Thymine.
- Analysis with cpptraj and curves+.

* Simulation time of 600 ns

[1] F. Beierlein, S. Volkenandt, P. Imhof, *J. Phys. Chem. B* **2022**, *126*, 1188-1201
[2] S. Volkenandt, F. Beierlein, P. Imhof, *Molecules* **2021**, *26*, 5728
[3] N. Kanaan, R. Crehuet, P. Imhof, *J. Phys. Chem. B* **2015**, *119*, 12365-12380
[4] L. S. Pidugu, *et. al.*, *J Am Chem Soc* **2019**, *141(47)*, 18851-18861
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Results

- N140D mutation destabilizes R275 interactions (figure 3).

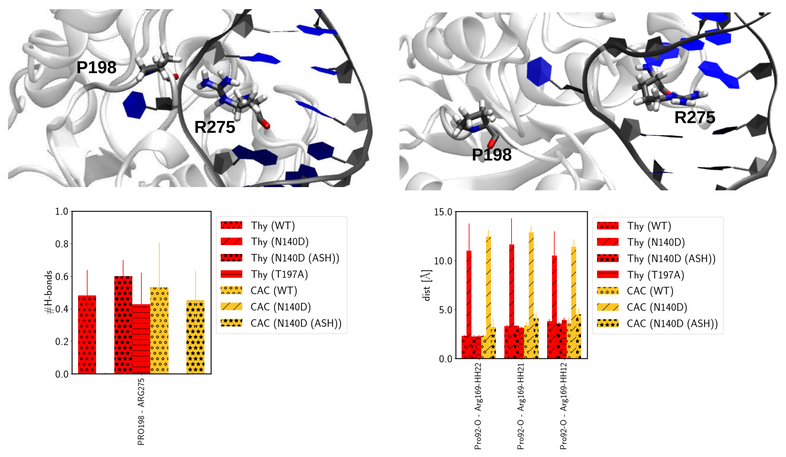

Figure 3. Upper left: Native conformation of TDG with R275 stabilizing the Protein-DNA complex. Upper right: Conformation of R275 in mutated N140D TDG. Lower left: Hydrogen bond formation between P198 and R275 in WT and mutated TDG simulations. Lower right: Distance between backbone carboxyl oxygen of P198 and the sidechain of R275

- Protein-DNA complex is destabilized in mutated TDG (figure 4).

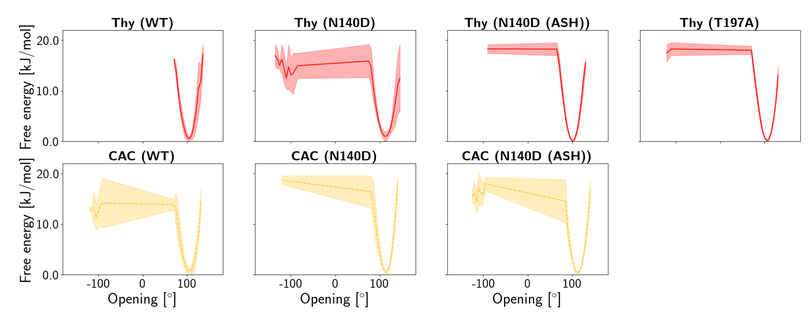

Figure 4. Free energy analysis of the opening angle of the DNA strand

- Hydrogen bond network in the active site is affected (figure 5).

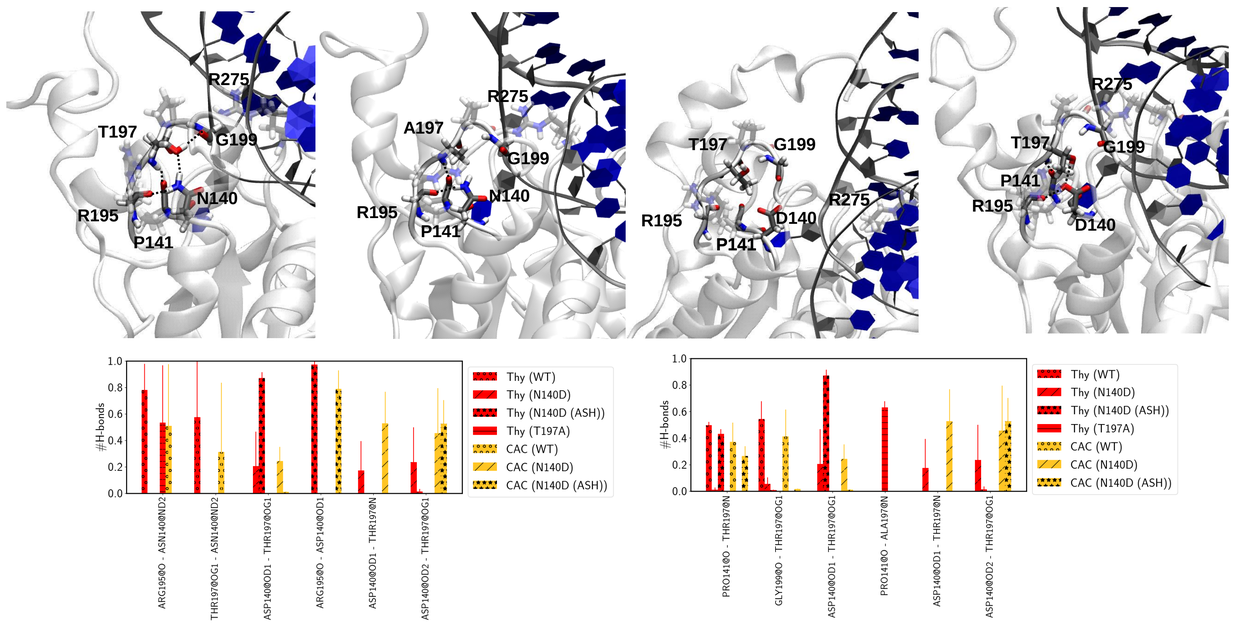

Figure 5. Top: Comparison of the hydrogen bond network around the active site between (from left to right) WT TDG, T197A TDG, N140D TDG (unprotonated) and N140D TDG (protonated). Bottom: Hydrogen network of X140 and X197 in WT and mutated TDG simulations.

Conclusion and Outlook

TDG activity is regulated via a network of hydrogen bond interactions. Mutations that affect these interactions reduce the enzyme DNA complex’s stability and hence the enzyme’s activity.
N140D is slightly active with caC as substrate, further work will be done to understand the specificity of N140D TDG.

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