Semiempirical calculations of *Thlaspi arvense* Thiocyanate Forming Protein (TFP) product formation

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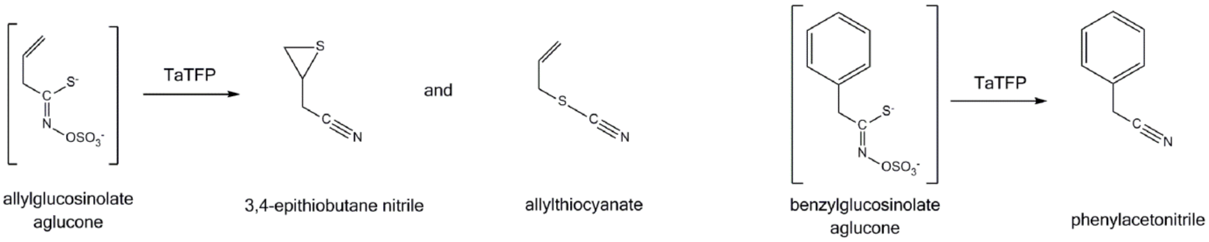
The crystal structure of *T. arvense* thiocyanate forming protein [1] is a representative of the so called specifier protein family which is involved in the glucosinolate degradation system in plants like mustard, horse radish, or broccoli. These specifier proteins exhibit a six bladed β-propeller structure with a closing blade which is formed by an N-terminal strand and three C-terminal strands. Extended loop structures are located on the bottom region of these proteins and form the active site. Based on the natural substrates allylglucosinolate aglucone and benzylglucosinolate aglucone, TaTFP forms thiocyanate, epithionitrile and nitrile, respectively.



The metal cofactor Fe^{2+} is octahedrally coordinated by amino acids of the highly conserved sequence motif EXXXDXXXH [2] and three water molecules. Protein ligand docking studies and semiempirical calculations of complexation energies indicate a substitution of one coordinating water with the negatively charged sulfur atom of the allylglucosinolate aglucone to form a stable low spin Fe^{2+} complex. Starting with this protein substrate interaction, a thermodynamically favored 3,4-epithiobutane nitrile formation was calculated with MOPAC [3]. An attack of the terminal double bond by the cleaved sulfur atom resulted in this three-membered ring structure. In the case of allylglucosinolate aglucone, the flexibility of TaTFP loop regions enables an additional metal substrate interaction with the terminal double bond of the allyl group. The electron pulling effect of the Fe^{2+} results in the thermodynamically favored formation of a stable allyl cation which subsequently can be attacked by the temporarily formed thiocyanate ion to form allylthiocyanate. Semiempirical calculations suggest that thiocyanate formation is only possible from this alternative substrate orientation.

[1] Gumz, F., et al., *Plant Mol Biol*, 2015. **89**(1-2): p. 67-81.
[2] Brandt, W., et al., *Plant Mol Biol*, 2014. **84**(1-2): p. 173-88.
[3] Stewart, J.J., J. *Comput. Aided Mol. Des.*, 1990. **4**(1): p. 1-103.