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COMPUTATIONAL MODELING OF 3'-PHOSPHOADENOSINE 5'- PHOSPHOSULFATE SYNTHASE PAPSS

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The sulfur nucleotide PAPS (3'-phosphoadenosine 5'-phosphosulfate) is the universal sulfuryl donor of the cell. In mammals 3'- phosphoadenosine 5'-phosphosulfate Synthase (PAPSS), using ATP, converts biochemically inert inorganic sulfate to the metabolically active PAPS. It is a bi-functional enzyme and catalyzes the formation of PAPS in two sequential steps [1]. In the first step, inorganic sulfate reacts with ATP to form APS and pyrophosphate. The resulting phosphoric-sulfuric anhydride bond has high energy that is the chemical basis of sulfate activation. The second step is catalyzed by the kinase domain of PAPSS and involves the reaction of APS with ATP to form PAPS and ADP. The proper function of PAPSS is essential for normal physiology in the human being. Although its overall mechanism and kinetics have been well studied in the past, more recent discoveries including the resolution of its crystal structure and research in its regulatory functions revealed previously unanticipated behaviors [2]. As the ubiquitous sulfate donor in most biological systems, the product of the enzyme, PAPS, plays an essential role in ECM formation, embryonic development and biomolecule secretion [3] Moreover, PAPSS has also been shown to be involved with the pathophysiology of a number of diseases including HIV, hepatocellular carcinoma and non-small cell lung cancer [4, 5, 6]. PAPSS deficiency in human results in osteochondrodysplasias or defective cartilage and bone metabolism as evidenced in the clinical condition of the recessively inherited, spondyloepimetaphyseal dysplasia (SEMD). Using a combination of homology modeling, molecular dynamics simulations and computational chemistry methods we try to understand how the three dimensional structure of PAPSS determines the enzyme function, focusing on the roles of specific amino acid residues/overall structures on the dynamics of the enzyme in aqueous solution and the related quaternary arrangements of the enzyme. Finally, enzymatic reactions are predicted/described in three-dimensional space and the reaction coordinate is explored through the lens of molecular dynamics simulations, hybrid QM/MM and quantum calculations. Results are discussed that give a realistic picture of the enzyme activity including molecular interactions, transition state structures and the reaction coordinate.

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1. Venkatachalam, K. Human 3'-phosphoadenosine 5'-phosphosulfate (PAPS) Synthase: Biochemistry, Molecular Biology and Genetic Deficiency. *IUBMB Life* **55**, 1–11 (2003).
2. Harjes, S., Bayer, P. & Scheidig, A. J. The Crystal Structure of Human PAPS Synthetase 1 Reveals Asymmetry in Substrate Binding. *J. Mol. Biol.* **347**, 623–635 (2005).
3. Venkatachalam, K. V., Akita, H. & Strott, C. A. Molecular Cloning, Expression, and Characterization of Human Bifunctional 3'- Phosphoadenosine 5'-Phosphosulfate Synthase and Its Functional Domains. *J. Biol. Chem.* **273**, 19311–19320 (1998).
4. Choe, H. & Farzan, M. in (ed. Enzymology, B.- M. in) 461, 147–170 (Academic Press, 2009).
5. Shih, W.-L. et al. Evidence for association with hepatocellular carcinoma at the PAPSS1 locus on chromosome 4q25 in a family-based study. *Eur. J. Hum. Genet.* **17**, 1250–1259 (2009).
6. Leung, A. W. Y. et al. 3'-Phosphoadenosine 5'-phosphosulfate synthase 1 (PAPSS1) knockdown sensitizes non-small cell lung cancer cells to DNA damaging agents. *Oncotarget* **6**, 17161 (2015).

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COMPUTATIONAL ENZYME DESIGN OF HALOALKANE DEHALOGENASES FOR YPERITE DEGRADATION

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Introduction: Bis-1-chloro-2-[(2-chloroethyl)sulfanyl]ethane, also known as yperite, is a blistering agent and a carcinogen causing nucleotide alkylation. Exposure to yperite leads to major skin, respiratory tract, and eye irritation [1, 2]. Enzymatic degradation of yperite offers many advantages over the traditional methods such as combustion or non-enzymatic chemical degradation. Enzymes can be used to decontaminate materials which would be otherwise destroyed by the chemical degradation, such as military or agricultural equipment [3]. Haloalkane dehalogenases, tested for enzymatic degradation, exhibit low catalytic efficiency, and thus low rate of degradation [3]. Here we describe computational re-design of three of these enzymes towards higher activity with yperite.

Methods: The binding modes of yperite in the active site of selected X-ray structures were obtained using the molecular docking. Subsequently, the minimized systems were analysed by quantum mechanic/molecular mechanic adiabatic mapping along the reaction coordinate of the S_N2 reaction. Using this method, transition state conformations were obtained for each system. Using the Rosetta Design [4], we have designed novel enzyme variants, which stabilize the transition state. The binding modes of yperite, thermodynamic parameters of the S_N2 substitutions, and thermostability of the novel variants were computationally predicted and compared to the wild-type structures. The relative occurrence of dehalogenation defined by the near attack conformation [5] was obtained by the molecular dynamics.

Results: Using these methods, we obtained 13 new designs which possess thermodynamically feasible mutations, a switch to an exothermic S_N2 displacement, much lower activation energy and a higher occurrence of the near attack conformation compared to their corresponding wild-type structures. Selected enzymes will be constructed and characterized experimentally. Novel enzymes re-designed towards higher catalytic activity with yperite could be used for decontamination and bioremediation.

1. M. Balali-Mood, M. Hefazi, *Fundam. Clin. Pharmacol*. **19** (2005) 297–315.
2. M. Doi, N. Hattori, A. Yokoyama, Y. Onari, M. Kanehara, K. Masuda, T. Tonda, M. Ohtaki, N. Kohno, *Am. J. Epidemiol.* **173** (2011) 659–666.
3. Z. Prokop, J. Damborsky, F. Oplustil, A. Jesenska, Y. Nagata, Patent WO 2006/128390.
4. F. Richter, A. Leaver-Fay, S.D. Khare, S. Bjelic, D. Baker, *PLoS ONE* **6** (2011) 1–12.
5. S. Hur, K. Kahn, T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.* **100** (2003) 2215–2219.