Computational Modeling of Sulfurylase Activity of Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase

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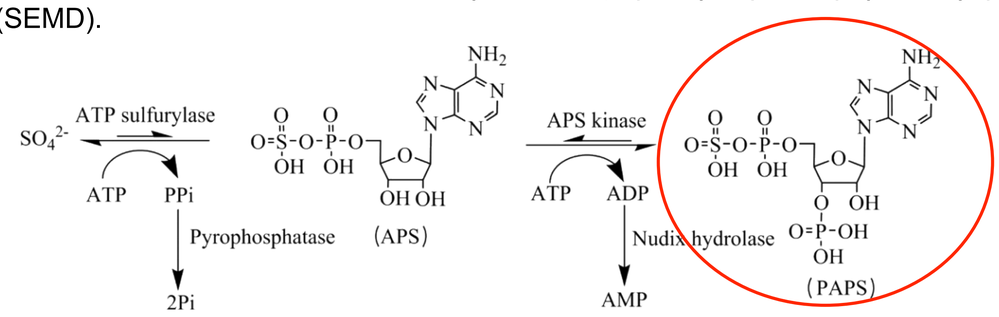
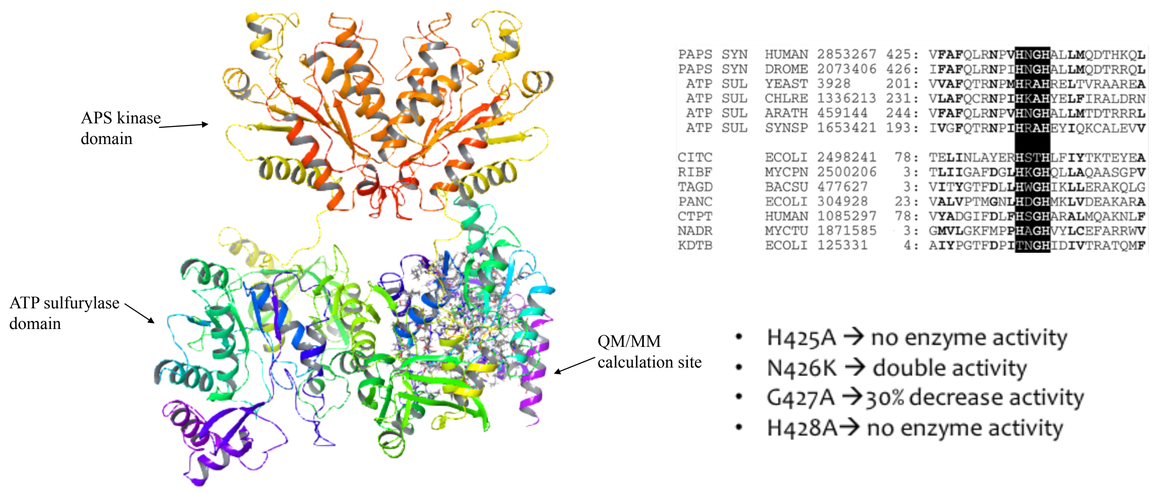
Introduction

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. In the first step, inorganic sulfate reacts with ATP to form APS and pyrophosphate. The resulting phospho-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. 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).



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PAPS SYN  HUMAN 2853267 425: VFAFQLRNPVHNGHALLMQDTHKQL
PAPS SYN  DROME 2073406 426: IFAFQLRNPIHNGHALLMQDTRRQL
  ATP SUL  YEAST 3928    201: VVAFQTRNPMHRAHRELTVRAARER
  ATP SUL  CHLRE 1336213 231: VLAFQCRNPIHKAHYELFIRALDRN
  ATP SUL  ARATH 459144  244: VFAFQLRNPVHNGHALLMTDTRRRL
  ATP SUL  SYNSP 1653421 193: IVGFQTRNPIHRAHEYIQKCALEVV

 CITC      ECOLI 2498241   78: TELINLAYERHSTHLFIYTKTEYEA
 RIBF      MYCPN 2500206    3: TLIIGAFDGIHKGHQLLAQAASGPV
 TAGD      BACSU 477627     3: VITYGTFDLIHWGHIKLLERAKQLG
 PANC      ECOLI 304928    23: VALVPTMGNIHDGHMKLVDEAKARA
 CTPT      HUMAN 1085297   78: VYADGIFDLIHSGHARALMQAKNLF
 NADR      MYCTU 1871585    3: GMVLGKFMPIHAGHVYLCEFARRWV
 KDTB      ECOLI 125331     4: AIYPGTFDPITNGHIDIVTRATQMF
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- H425A → no enzyme activity
- N426K → double activity
- G427A →30% decrease activity
- H428A→ no enzyme activity

Human 3'-phosphoadenosine 5'-phosphosulfate Synthase

Top: *Reaction diagram of sequential reaction of PAPS synthase.*
Bottom left: *Crystal structure of PAPS synthase with functional domains and QM/MM calculation site in sticks.*
Bottom right: *Conserved HXGH motif in sequence alignment*

Methods

Since crystal structures of human PAPSS1 with various ligands and combinations have been resolved by X-ray diffraction to a resolution of up to 1.75 Å (PDBs: 1x6v, 2ofw, 1xjq, 1xnj), atomistic molecular dynamics simulations and ligand-docking can be applied to gain an understanding of the structural and functional consequences of specific residues in the conserved HXGH motif that is part of the substrate binding pocket of the sulfurylase domain. Mutations in this motif have been studied experimentally and reported to alter the enzyme function, however, no molecular explanation has been reported so far. Homology modeling in YASARA allowed modeling of unresolved loops and *in silico* mutagenesis, ligand docking was performed in AUTODOCK and all complex structures were simulated using molecular dynamics simulations in GROMACS for at least 100ns using the AMBER99SB force field. MM-PBSA calculations were performed to calculate free energies. QM/MM method using *Schrödinger Inc.*'s QSite package is applied to model the reaction mechanism in sulfurylase domain. The reaction coordinate is modeled both in the forward and reverse direction. QM calculation site involves only the ligands and MM site includes all atoms within 10 angstroms.

Objectives

1.Understanding the reaction mechanism of ATP sulfurylase reaction by finding the theoretical transition structure.
2.Elucidate the roles of critical residues within the active site.
3.Creating a potential energy profile for the reaction coordinate.

Mm-pbsa method:
Energy Calculations
molecular mechanics energies combined with the Poisson–Boltzmann surface area

	WT (ATP) kJ/mol	G427A (ATP) kJ/mol	N426K (ATP) kJ/mol	N426K(ATP)-alternative K kJ/mol	H425A_ H428A (ATP) kJ/mol	WT (APS) kJ/mol
Van der Wall energy	-158 +/- 19.298	-165.19 +/- 13.77	-154.449 +/- 12.773	-189 +/- 20.299	-177.842 +/- 17.056	-206.016 +/- 15.636
Electrostatic energy	-351 +/- 48.852	-86.049 +/- 27.306	-386.018 +/- 47.131	-554 +/- 55.881	230.494 +/- 76.231	-221.110 +/- 38.228
Polar solvation energy(est)*	488	488	488	488	488	488
SASA energy	-17.85 +/- .458	-18.647 +/- .657	-17.181 +/- .627	-19.752 +/- 0.824	-17.490 +/- 1.141	-16.714 +/- .519
Binding energy	**-40**	**218**	**-70**	**-274**	**524**	**44**

References

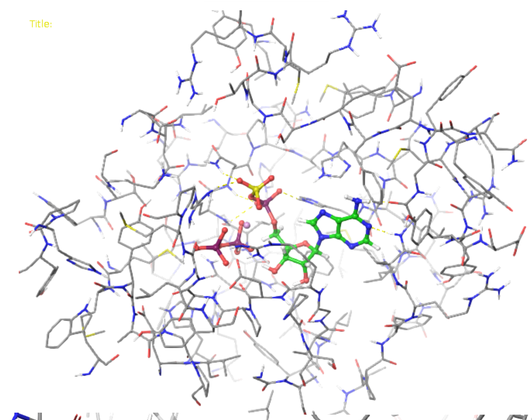
Sekulic, N., Dietrich, K., et al. (2007). Elucidation of the Active Conformation of the APS-Kinase Domain of Human PAPS Synthetase 1. Journal of Molecular Biology, 367(2), pp.488-500.

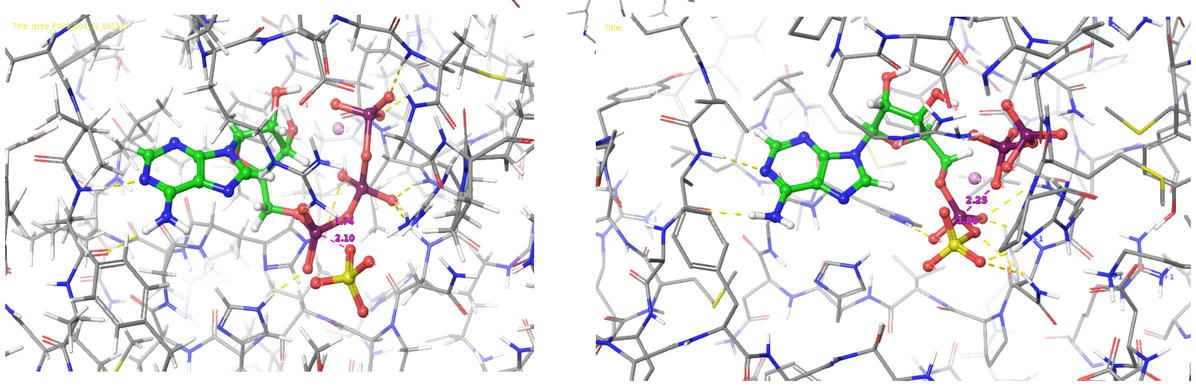
Strott, C. (2002). Sulfonation and Molecular Action. Endocrine Reviews, 23(5), pp.703-732.

Venkatachalam, K. (2003). Human 3′-phosphoadenosine 5′-phosphosulfate (PAPS) Synthase: Biochemistry, Molecular Biology and Genetic Deficiency. IUBMB Life (International Union of Biochemistry and Molecular Biology: Life), 55(1), pp.1-11

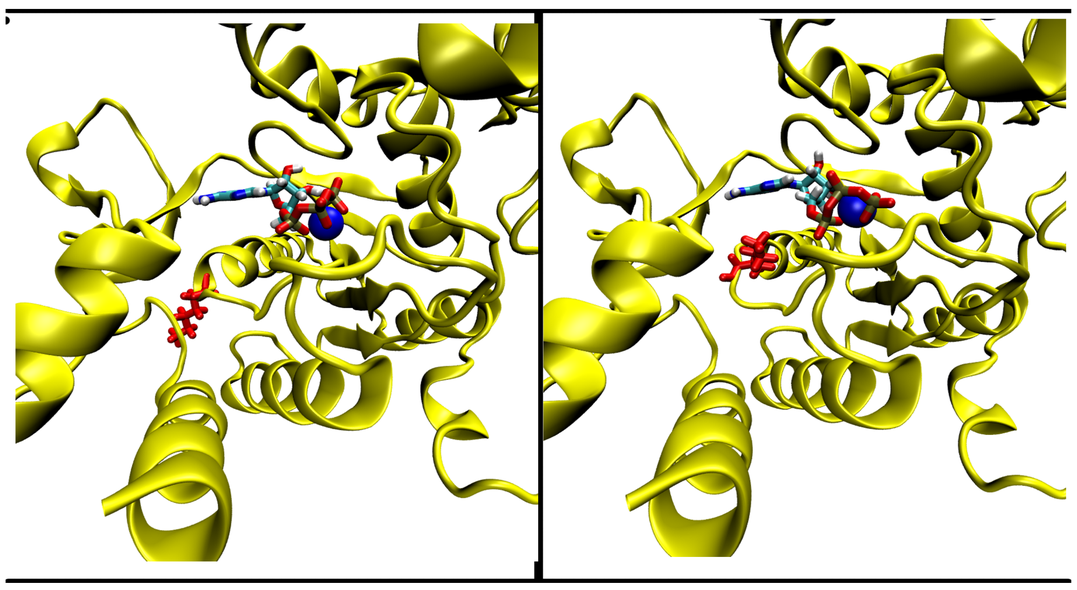
Venkatachalam, K., Fuda, H., et al. (1999). Site-selected Mutagenesis of a Conserved Nucleotide Binding HXGH Motif Located in the ATP Sulfurylase Domain of Human Bifunctional 3'-Phosphoadenosine 5'-Phosphosulfate Synthase. Journal of Biological Chemistry, 274(5), pp.2601-2604.

Results

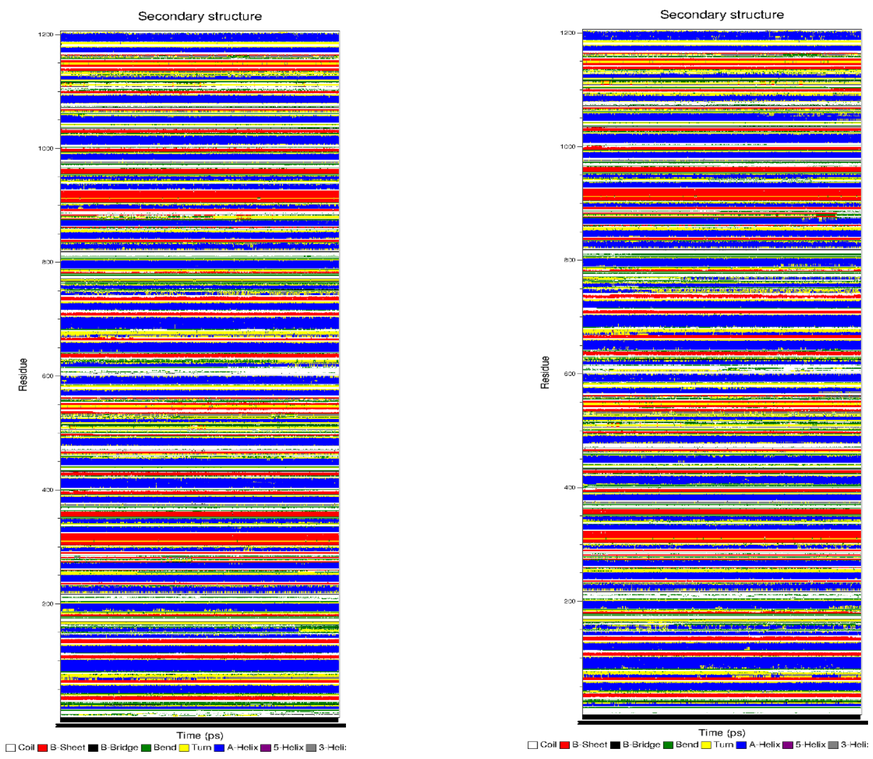




QM/MM region for QSite calculation and approximate transition structures



Alternative Conformation of N426K in Sulfurylase Domain



Comparison of Secondary structure prediction in alternative conformation of N426K mutant simulations

Conclusions

1.In QM/MM calculation Molecular scan followed by step-wise QM/MM minimization revealed an S_N1 type mechanism with trigonal planar intermediate. However, this result might be the artifact of constrained search and needs to be confirmed with Hessian Eigenvalue analysis.

2. Alternative conformations of both N426K mutant simulation keep similar secondary structure through out the simulation.

3. Binding energies correlate with the experimental results obtained. While both histidine residues are contacting ATP and point mutations do affect substrate binding directly, the effect of point mutations in 426 (G to A) and 427 (N to K) is rather indirect as those residues do not contact ATP. Altered protein dynamics as a results of the mutations propagate into the active site and result in a lower binding affinity for G427A. Interestingly a higher binding affinity is observed for N426K, correlating well with the reported increased enzymatic activity in *in vitro* experiments and in alternative conformation of Lysisine 426 increases the affinity significantly.

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