Structural insights into *E. coli* porphobilinogen deaminase during synthesis and exit of 1-hydroxymethylbilane

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**INTRODUCTION**

- Porphobilinogen deaminase (PBGD) catalyses the formation of 1-hydroxymethylbilane (HMB), a crucial intermediate in tetrapyrrole biosynthesis, through a step-wise polymerisation of four molecules of porphobilinogen (PBG), using a unique dipyrromethane (DPM) cofactor [1]
- Previous studies suggested residues of catalytic importance [2,3], but their role in the catalytic mechanism and dynamics of the protein is unknown
- *E. coli* PBGD was chosen as a model system to understand the enzyme mechanism and protein dynamics during pyrrole chain elongation and product exit

**METHODOLOGY**

- Structure of *E. coli* PBGD taken from PDB - 2YPN; missing residues 43 to 59 were loop modeled using Modeller [9]
- Pyrrole Chain elongation
  - Stages of simulation - DPM, P3M, P4M, P5M and P6M
  - Force field parameters for the pyrrole moieties were obtained from ATB server [4]
  - 35 ns explicit solvent MD simulations were performed using Gromacs 4.5.5 with G5Sa6 force field for each stage
- Exit Mechanism
  - Steered MD (SMD) simulations were carried out, to study probable exit paths of HMB from the enzyme
  - Initial guess of directions for SMD were based on CAVER [5], a pymol plugin, to find channels
  - HMB was pulled at a constant velocity of 1 Å/ns with an integrating time step of 1 fs
  - MD run for 150 ns was performed on protein with cofactor (DPM) after removal of HMB

**CONCLUSION**

- Compactness of the overall protein decreased with domain 1 and domain 2 moving away from each other, re-adjusting themselves for the growing pyrrole chain
- The cofactor turn region, moves into the active site cleft with an inclinination towards domain 2
- D50, K55 and R149 has role in active site loop modulation
- The possible exit path for HMB is through the interface between domain 1, domain 2 and activite site loop
- Compactness of the protein in post-SMD simulation gradually increases indicating its ability to regain its initial stage conformation and subsequent catalytic role
- R11, Q19 and R176 have a role in product exit along with being catalytically important

**REFERENCES**


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