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]. Though residues of catalytic importance were suggested [2, 3], their role in the catalytic mechanism and dynamics of the enzyme is not known.

*E. coli* PBGD was used 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 was taken from PDB (2YPN), and the missing coordinates of the loop (43 to 59) were modeled using Modeller9v8.
- 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 molecular dynamics (MD) simulations were performed using Gromacs 4.5.5 with G533bf 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 (No-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, moves into the active site cleft with an inclination towards domain 2.
- D50, K55, and R149 has role in active site loop modulation.
- The possible exit path for HMB is through the space between domain 1, domain 2, and active site loop.
- Compactness of the protein in No-HMB simulation gradually increases indicating its ability to regain its initial stage conformation, and subsequent catalytic role.
- Catalytically important residues R11, Q19, and R176 also have a role in product exit.

**REFERENCES**


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