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| Title: | In silico studies of structural and biochemical properties of human cystathionine β-synthase (HCBS) enzyme |
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| Abstract: | Human cystathionine β-synthase (hCBS) is a unique, Heme containing, and pyridoxal 5'-phosphate (PLP) dependent enzyme which plays a pivotal role in the transsulfuration pathway. Homocystinuria is a common disease caused by mutation or deficiency of hCBS enzyme. The hCBS enzyme consists of three domains; viz. PLP bound catalytic domain, Heme bound N-terminal domain and C-terminal domain. The PLP catalytic center forms an internal aldimine via the formation of Schiff base with Lysine (Lys119) and is bound to the α-helix 8 through the hydrogen bonds. PLP-Lys119 internal aldimine catalyzes the condensation of homocysteine with serine. The hexacoordinated Heme is axially bound with Cysteine (Cys52) and Histidine (His65) residues. Heme resides almost ~20Å apart from the PLP site but influences the overall enzymatic activity of hCBS. Heme and PLP communicate with each other via the α-helix 8. Although the exact nature of Heme's association with PLP is unknown, absence of Heme in the hCBS, reduce the enzymatic activity. On the other hands the Sadenosyl methionine (SAM) acts as an allosteric regulator upon binding with C-terminal domain for the enzyme activations. SAM binding modulates the enzyme conformation; converting the basal to the active conformation. Despite of large experimental studies primarily on the crystal structures of the enzymes and its activities, the exact understanding behind the long-range allosteric communication between PLP and Heme is still unknown. To unravel this, we performed atomistic molecular dynamics simulations in conjunction with density functionals theory (DFT). Performing classical molecular dynamics (MD) simulation for systems containing hexacoordinated Heme (FeIII) is challenging due to lack of specific force field parameters in the literature. Thus, in the beginning of the project, the required force fields were explicitly developed. In case of Cys52 and His65 bound hexa-coordinated Heme, we developed it adopting a number of DFT based hybrid functionals. To access the quality of the developed force fields the rigorous benchmarking procedures were adopted by comparing the dynamics as obtained from the force field parameter using different DFT functionals and compared them with available experimental parameters. The simulation studies revels that the hydrogen bond between the Heme and α-helix 8 via Cys52- Arg266 interactions play a crucial role in electron transfer process from Heme to the catalytic centers. We confirm the role of this specific hydrogen bond upon R266K mutation studies that has already experimentally proved to reduce the enzyme activities. This single mutation imparts a large structural effect leading to disruption of the stable structure and rigidity in the protein. R266K mutation also diminishes the electronic communication between Heme and PLP region and it also inhibit the substrate channeling at the catalytic center. Next, we have investigated the role of α-helix 8 in the electron transport channel between Heme and PLP. The α-helix 8 is bound to Heme with a single hydrogen bond while it is bound with PLP cofactors through multiple hydrogen bonds. In the ground state we realized that α-helix 8 accumulates electrons from both of these co-factors. This observation provides a new perfective to the Heme-PLP communications that was believed to the bi-directional in nature. The α-helix 8 in principle acts as the electron reservoir for the enzymatic activities of the PLP centers. We further realized that the multiple-hydrogen bonds between α-helix 8 and PLP is the key player to module the electron density at the Schiff base carbon atom that initiate the condensation reaction i.e., the enzymatic activities. The key observations of the electron-delocalization at the ground state have further been confirmed from excited state calculations of absorption spectrum applying sTD-DFT methodology. The later studies reveled the long-range electronic overlap between Heme and PLP. In the final part of this project, we have investigated the activation of the CBS enzyme upon binding it with the SAM molecules. The molecular dynamics studies for the basal and active conformations and their projections along with the principal components reveal the most probable SAM binding sites in CTD and the intermediate states. These provide various crucial understandings of the enzyme activations. |
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