Poster Presentation

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Communication between the two catalytic domains of Formylglycinamide ribonucleotide amidotransferase

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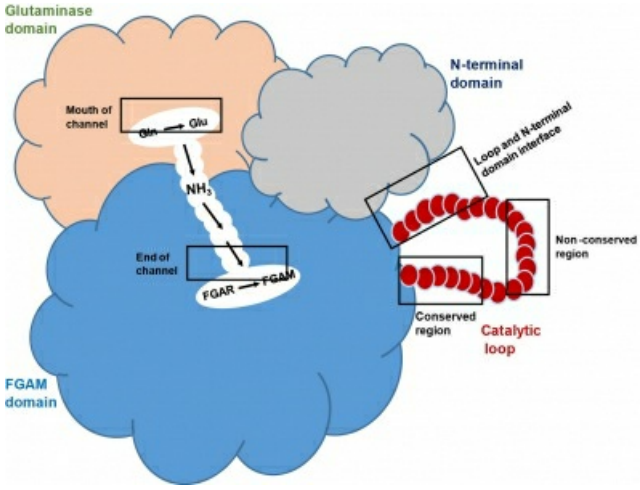
Purine metabolic pathway, a highly conserved pathway in various organisms, provides purine nucleotides for the synthesis of DNA and RNA. Formylglycinamideribonucleotide amidotransfearse (FGAR-AT) from Salmonella typhimurium encoded by PurL gene (StPurL) is a 143 kDa multi-domain, bifunctional enzyme which catalyzes the fourth step of purine biosynthesis and is characterized by a cys-his-glu catalytic triad present in its glutaminase domain. This domain is responsible for glutamine to glutamate conversion and the released ammonia travels through an intra-molecular pathway to the other active site in FGAM synthetase domain thereby converting FGAR to FGAM. Though its crystal structure and the pathway releasing ammonia from one active site to another, has been determined, the communication mechanism between the two functional domains remains elusive. In the present work, structural and mutational studies have been performed at the regions proximal to the glutaminase active site, where ammonia is produced, at the end of the channel, where it is consumed and in the catalytic loop of the FGAM synthetase domain. Surprisingly, results indicate that certain mutations like R1263A and S1052D exhibit a 15 to 25% increased in overall catalysis. Furthermore, the crystal structure of the R1263A mutant exhibits conformational changes 25 Å away, near the FGAM active site, asserting its regulatory role in catalytic communication. The perturbations introduced at the end of the channel like T310N and G313S, showed drastic loss in FGAM production leading to the uncoupling between the two activities. Crystal structure of T210N reveals that this may happen because of constriction introduced at end of the channel blocking ammonia from reaching the active site. In addition, mutagenesis and shortening of the structurally disordered FGAM catalytic loop reveals that the interactions of this dynamic loop with the N-terminal connector domain have a profound effect on the distal glutaminase activity. Therefore, it appears that a relay mechanism is triggered by the conformational changes in the catalytic loop that transmits signal for the progress of the reaction from one active site to another.

[1] Anand, R. et al. (2004). Biochemistry 43, 10328-10342.
[2] Tanwar, A.S. et al. (2012). Acta Crystallographica Section D 68, 627-636.
[3] Zhang, Y. et al. (2008). Cellular and Molecular Life Sciences 65, 3699-3724.



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