Regulation of primary and secondary reactions of nitrilase

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Abstract

Enzyme-catalyzed reactions often undergo a multiple reaction path, so the reaction products often have the problem of diversity. It will increase the difficulty of separation and purification in industrial applications. Some protein engineering techniques such as directed evolution can adjusted the enzyme reaction to one direction but the efficacy is low. In this study we used a novel *in silico* computational protocol to solve the problem. Nitrilase shows nitrilase activity and nitrile hydratase. We discovered the mechanism of which is that the protonation competition between the NH2 group from the cyano group of the substrate and the SG of catalytic Cys in the enzyme-bound tetrahedral reaction intermediate regulated the reaction pathways of nitrilase. Then the QM/MM calculation was applied to explore the controversial aspects of nitrilase catalytic mechanism. The proton of nitrilase transferred to the Lys135 from the hydroxyl group of substrate-bound tetrahedral reaction intermediate, and then transferred to Glu53, and the acid product finally formed as the proton transferred to the substrate from Glu53. By means of QM/MM simulation, hydrolysis process of nitrilase was analysed for the first time, providing the theoretical support for the follow-up researchers. Several mutations were designed by QM/MM and NAC calculation, and experiment proved that both nitrilase and nitrile direction can be controlled easy.

Brief Biography

Zhang received his B.S. in Pharmaceutical Microbiology and M.S. in Biochemical Engineering from Nanjing Tech University, and his Ph.D. in Biochemistry and Molecular Biology from East China University of Science and Technology. He was trained as a Postdoctoral Researcher at California Institute of Technology (Caltech) in the lab of Prof. Grant J. Jensen (HHMI). Zhang's research interests are structure, function, and the mechanism of proteins, as well as the rational design of enzymes for industrial applications. With a combination of experiments and computation, he has discovered the structures (by crystallography) and the mechanisms of some important enzymes just like lipase, oxidordeuctase, and nitrilase. He has also developed many computational methods for the rational design of proteins, for example, the optimization of protein thermal stability based on charge-charge interaction refinement, changing coenzyme selectivity based on hydrogen bond reconstruction, and switching the enzyme production preferring based on

the design of reaction pathway. Also, he focused on the structures (by Cryo-EM) and interactions of multi protein biological macromolecules, including proteases and their protein substrates and GPCRs. In recent years, he has published more than 40 SCI papers in reputational journal including *Catalysis Science Technology, Applied Environmental Microbiology, Biotechnology for Biofuels, food chemistry.* He also obtained The First Prize of Shanghai Science and Technology in twice.