MS21.O05

Lysine carbamylation for enzymatic function: metal and structural requirements

Y. Hsieh[1], S. Chan[2], Y. Yang[3], C. Chen[1]

[1]National Synchrotron Radiation Research Center, Life Science Group, Hsinchu, Taiwan, [2]California Institute of Technology, Division of Chemistry and Chemical Engineering, Pasadena, USA, [3]National Chiao Tung University, Department of Biological Science and Technology, Hsinchu, Taiwan

Lysine carbamylation, a post-translational modification, facilitates metal coordination for specific enzymatic activities. Carbamylation on lysine extends the residue length by ~2 Å and changes the side chain from a positive to negative charge at neutral pH. The proteins involved with lysine carbamylation are found to be related to the human diseases, such as type 2 diabetes, developmental delay, metabolic acidosis, mental retardation, hypotonia and seizures. We have determined structures of the vertebrate dihydropyrimidinase from Tetraodon nigroviridis (TnDhp) in various states: the apo enzyme as well as two forms of the holo enzyme with one and two metals at the catalytic site. The essential active-site structural requirements have been identified with possible existence of four metal-mediated stages of lysine carbamylation. Only one metal is sufficient for stabilizing lysine carbamylation; however, the post-translational lysine carbamylation facilitates additional metal coordination for the regulation of specific enzymatic activities through controlling the conformations of two dynamic loops, Ala69–Arg74 and Met158–Met165, located in the tunnel for the substrate entrance. The substrate/product tunnel is in the “open form” in the apo-TnDhp, in the “intermediate state” in the mono-metal TnDhp, and in the “closed form” in the di-metal TnDhp structure, respectively. Structural comparison also suggests that the C-terminal tail plays a role in the enzymatic function through interactions with the Ala69–Arg74 dynamic loop. In addition, the structures of the di-metal TnDhp in complexes with hydantoin, N-carbamyl-β-alanine and N-carbamyl-β-amino isobutyrate, as well as apo-TnDhp in complex with a product analog, N-(2-acetamido)-iminodiacetic acid, have been determined. These structural results illustrate how a protein exploits unique lysines and the metal distribution to accomplish lysine carbamylation as well as subsequent enzymatic functions.

[1] *Y.-C. Hsieh, M.-C. Chen, C.-C. Hsu, et al., J. Biol. Chem. 2013, 288, 30645-30658,* ***[2]*** *C.-Y. Huang C.-Y., C.-C. Hsu, M.-C. Chen et al., J. Biol. Inorg. Chem. 2009, 14, 111-121*

Keywords: lysine carbamylation, post-translational modification, dihydropyrimidinase