Poster Presentation

MS001.P14

Structural and functional studies of Vibrio cholerae c-di-GMP phosphodiesterase

Malti Yadav[1], Udayaditya Sen[1]
[1]*Crystallography And Molecular Biology, Saha Institute Of Nuclear Physics, Kolkat, Kolkata, India*
E-mail: malti.yadav@saha.ac.in

Cyclic dimeric GMP (c-di-GMP) is a global second messenger which controls a range of different cellular functions in bacteria at transcriptional, translational, and post-translational level. c-di-GMP binds to various receptor proteins or riboswitches and regulates biofilm formation, motility, cell cycle progression and virulence in bacteria (2). The intracellular level of c-di-GMP is controlled by the opposing action of two different groups of enzymes. The diguanylate cyclases containing the GGDEF domain produce c-di-GMP from two molecules of GTP, whereas specific phosphodiesterases (PDEs) associated with EAL or HD-GYP domains hydrolyze the cyclic molecule c-di-GMP to linear 5-pGpG, which is subsequently hydrolyzed into two GMP molecules (1,2) . The increase in intracellular c-di-GMP concentration leads to biofilm formation while its decrease enhances bacterial mobility and virulence.

Here, we discuss the biochemical and structural aspects of EAL domain protein from Vibrio cholerae O395 (VcEAL). In Vibrio cholerae, EAL domain plays a crucial role in biofilm dispersal by reducing the c-di-GMP concentration in the cell, thereby inhibiting cholera pathogenesis and disease transmission by making it susceptible to antibiotic treatment. VcEAL was overexpressed and purified and high resolution crystal structures of EAL domain in complex with its substrate c-di-GMP and metal ions involved in catalysis or in enzyme inhibition at different pHs (1.95 Å, 2.3 Å) or in apo form (2.4Å) were determined. Apo and holo VcEAL structures looks very much similar indicating there is subtle change upon c-di-GMP binding. Comparing c-di-GMP/EAL interactions coupled with multiple sequence alignment revealed 12 conserved residues in the active site which modulate c-di-GMP binding/release. Of these 12 residues, 7 residues (mostly Glu or Asp) interact directly with c-di-GMP through two metal ions. Two oxygen atoms of the c-di-GMP and potential catalytic water molecules in the active site complete the coordination. Through kinetic studies we demonstrate that VcEAL is a potent phosphodiesterase. The phosphodiesterase activity is optimal at alkaline pH and strongly dependent upon Mg2+ or Mn2+ whereas Ca2+ inhibits the reaction. We have generated a set of mutants and assessed their enzymatic activity the results of which will also be presented.

1. Schmidt, A. J. et al. (2005). J. Bacteriol. 187, 4774–4781.
2. Tchigvintsev, A. et al. (2010). J. Mol. Biol. 402, 524–538.

Keywords: Vibrio cholerae, EAL domain, c-di-GMP