Poster Presentation

MS37.P01

Crystal structures of LsrR complexed with p-AI-2 reveal distinct mechanisms

J. Ha[1,2], E. Kim[1], H. Cheong[1], K. Ryu[1,2]

[1]Korea Basic Science Institute, Division of Magnetic Resonance, Ochang, South Korea, [2]University of Science and Technology, Department of Bio-Analytical Science, Daejon, South Korea

Quorum sensing (QS) is a cell-to-cell communication system and responsible for a variety of bacterial phenotypes including virulence and biofilm formation. QS is mediated by small molecules, autoinducers (AIs), including AI-2 that is secreted by both Gram (+/−) microbes. LsrR is a key transcriptional regulator that governs the varied downstream processes by perceiving AI-2 signal, but its activation via autoinducer-binding remains poorly understood [1]. The ligand-free crystals of LsrR and complex crystals of LsrR and C-LsrR with 5 mM R5P were grown with reservoir buffer. Complex crystal of C-LsrR/D5P and C-LsrR/D8P were obtainded by soaking the native crystals in the same crystallization buffer (pH 6.5, 0.1 M bis-tris, 9.1% PEG-3350, 10 mM barium chloride dehydrate, 10 mM R5P) containing 0.15mM D5P and 2.0 mM D8P. These crystals were determined its 3-demensional (3D) structure at 3.2 Å ~ 1.9 Å resolution after SAD phasing. The ligand-binding affinities for LsrR protein were measured using fluorescence spectrophotometer and Isothermal titration calorimetry (ITC) while increasing the ligand concentrations. Detailed regulatory mechanism of LsrR from the crystal structures in complexes with the native signal (phospho-AI-2, D5P) and two quorum quenching antagonists (ribose-5-phosphate, R5P; phosphoisobutyl-AI-2, D8P). The bound D5P and D8P molecules are not the diketone forms but rather hydrated, and the hydrated moiety forms important H-bonds with the carboxylate of D243. The D5P-binding flipped out F124 of the binding pocket, and resulted in the disruption of the dimeric interface-1 by unfolding the α7 segment. However, the same movement of F124 by the D8P'-binding did not cause the unfolding of the α7 segment. Although the LsrR-binding affinity of R5P (Kd, ~1 mM) is much lower than those of D5P and D8P (~2.0 and ~0.5 μM), the α-anomeric R5P molecule fits into the binding pocket without any structural perturbation, and thus stabilizes the LsrR tetramer. The binding of D5P, not D8P and R5P, disrupted the tetrameric structure and thus is able to activate LsrR. The detailed structural and mechanistic insights from this study could be useful for facilitating design of new anti-virulence and anti-biofilm agents based on LsrR.

[1] *C. M. Waters, B. L. Bassler, Annu. Rev. Cell Dev. Biol. 2005, 21, 319-46*

Keywords: quorum sensing, repressor protein, signal molecules, AI-2, X-ray crystallography