Poster Presentation

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Study of a TCS activated by light in Brucella abortus

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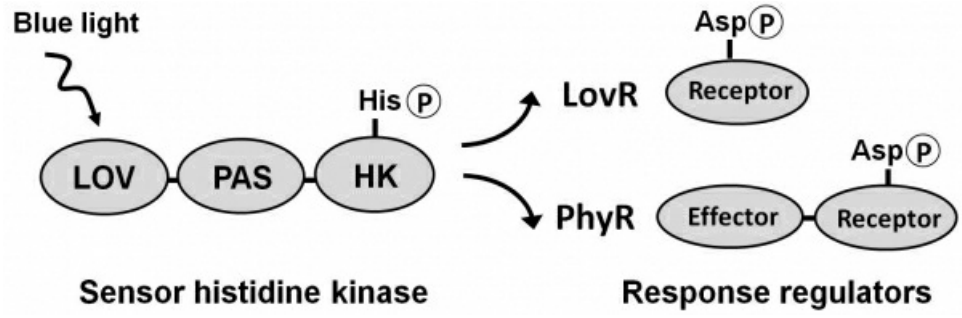
Two-component signal transduction systems (TCSs) are modules that allow bacteria to rapidly adapt to changing environmental conditions. In the most common case, they are formed by a sensor histidine kinase (HK) which, upon sensing of an external signal, autophosphorylates at a conserved histidine residue and then transfers the phosphoryl group to a conserved aspartate residue in a cognate response regulator (RR). The latter protein undergoes structural changes that are able to modify gene expression by directly binding to DNA, catalyze metabolic reactions or alter protein-protein interactions. The pathogenic bacterium Brucella abortus, the causative agent of the disease brucellosis, bears a particular two-component system formed by a dimeric cytoplasmic three-domain blue-light sensor HK (LOV-PAS-HK) and two monomeric RRs called PhyR and LovR. The activation of this HK has been shown to increase the virulence of this pathogen. With the goal of understanding at the atomic level the activation and signal transduction events of this system, we aimed to solve the three-dimensional structures of these proteins by means of X-ray diffraction. The core of the blue-light sensor FMN-binding LOV domain was crystallized and its structure solved at 1.64 Å resolution in the dark. It adopts the alpha/beta PAS domain fold and presents a hydrophobic central beta-scaffold that interacts in one face with an FMN molecule and in the other with a neighboring monomer forming an unexpected antiparallel homodimer. This beta-scaffold destabilizes upon light exposure and therefore was proposed as a key element in the signal transduction mechanism [1]. Interestingly, we were also able to determine the structure of a construct comprising the LOV core domain plus an N-terminal capping helix at 2.34 Å resolution (N-LOV), observing now the expected parallel dimerization nature of the protein. This structure let us understand at the atomic level the important contribution of this N-terminal element in the stabilization of the quaternary structure and its photochemical behavior. This parallel arrangement has been recently confirmed with the determination of the N-LOV-PAS structure at 2.74 Å resolution, which holds a long connecting alpha-helical element between both globular domains. Additionally, we were able to solve the crystal structure of the isolated HK domain at 2.51 Å resolution by sulfur SAD in a challenging procedure, due to low sequence identity available models for MR, the low symmetry P2(1) space group present and the existence of four copies of the molecule in the 108-kDa asymmetric unit (AU) [2]. Interestingly, the HK structure presents two different dimeric assemblies in the AU, which allowed us to propose a mechanism of activation [3]. To finish, we were also able to determine the structure of the PhyR RR at 2.05 Å resolution. Efforts are underway to obtain the structure of the full LOV-PAS-HK protein as well as HK-RR complexes. All these protein structures, together with spectroscopic, activity and biophysical assays, allowed us a better understanding of this crucial system for the pathogenicity of Brucella.

[1] Rinaldi, J. et al. (2012). J. Mol. Biol. 420, 112-127.
[2] Klinke, S. et al. (2015). Acta Cryst. D71, 1433-1443.
[3] Rinaldi, J. et al. (2016). J. Mol. Biol. 428, 1165-1179.



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