CheA-CheY Interactions in a Two-Component Signal Transduction System

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Abstract

Many bacteria respond to nutrient gradients by controlling the rotation of the flagella motor. Bacteria such as *Escherichia coli* sense environmental cues using a chemotaxis signal transduction system consisting of a kinase sensor, CheA and a response regulator, CheY. CheA undergoes autophosphorylation on a histidine residue and the phosphate group is in turn transferred to an aspartate residue on CheY. This study focuses on how CheA and CheY interact in order to provide correct orientation for the transfer of the phosphate group. CheA-CheY interactions have been elucidated by new NMR structures. The P1 and P2 functional domains located at the N-terminal region of CheA are involved in binding CheY. The P2 docking domain binds CheY with high affinity. Mutational analysis showed that Phe214 of the P2 domain is critical for high affinity binding. NMR analysis of the CheA-CheY complex revealed specific low affinity binding between CheY and P1. The interaction surface was predominantly with the first half of the α1 helix of P1. Phe8 and Glu15 of the α1 P1 play a role in specific binding of CheY to P1 CheA, as the rate of phosphorylation is 5-30 times slower when these residues have been mutated and rendered non-functional. These results refine our understanding of the structural features involved in CheA-CheY interactions and helps us to better appreciate the molecular mechanism of the phosphotransfer between the sensor kinase CheA and flagella motor-binding protein, CheY.

Introduction

Cells use signal transduction pathways to sense and respond to environmental and developmental cues. Flagellated bacteria like *Escherichia coli* use a chemotaxis signal transduction system to swim towards attractants or away from repellants. In absence of attractant the flagella rotate clockwise causing the bacterium to tumble. In the presence of attractant the motor rotates counter-clockwise causing directed swimming into the gradient. Bacteria employ two-component signal transduction systems consisting of a histidine kinase and a response regulatory protein to sense extracellular signals. The histidine kinase undergoes autophosphorylation on a histidine residue and the phosphate group is transferred to an aspartate residue on the response regulator. Typically the response regulator is a DNA binding protein that regulates gene expression. The two-component chemotaxis system differs from other systems in that CheA, the histidine kinase, is bound to a separate sensor protein through a coupling protein CheW. Phosphorylation of the response regulator, CheY, induces interaction with the FliM protein of the motor rather than binding to DNA. CheA is functionally a homodimer containing five domains. In this study the structural and functional interactions between CheA and CheY are examined focusing on the P1 domain that contains the site of phosphorylation (His 48) and the P2 domain that binds CheY with high affinity.

Two-Component Signal Transduction System

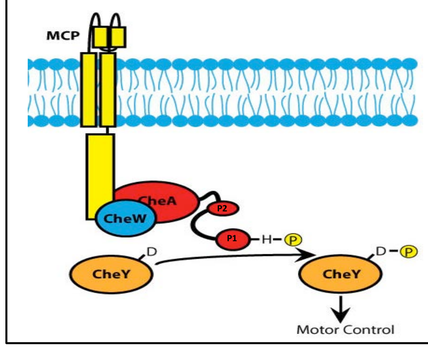

Figure1: Methyl-accepting chemotaxis proteins (MCP) are embedded in the inner membrane, sensing the environment. CheA is communicates with MCP through interactions with CheW. When no signal is present CheA autophosphorylates and passes the phosphate to CheY. Once phosphorylated, CheY is able to interact with the flagellar motor switch causing the flagella to rotate clockwise and the bacteria to tumble.

Functional Domains of CheA and CheY

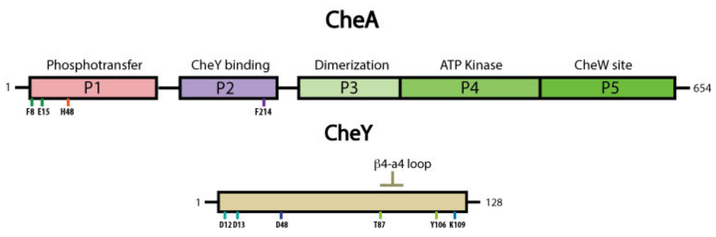

Figure 2: CheA consists of five domains (P1-P5). Domains in green are not included in the model. P1 and P2 domains of CheA and CheY are color coded to match the model. P2 domain of CheA has the highest affinity for CheY. A transfer of a phosphate molecule from His48 of P1 to Asp57 of CheY stabilizes the active conformation of CheY. Additional markings represent residues that are important for other functions discussed later.

CheA Docked to CheY

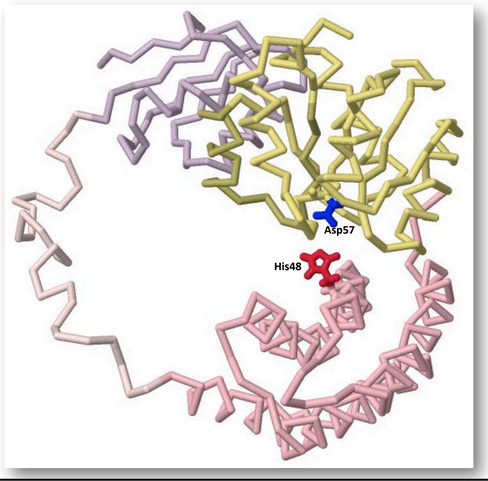

Figure 3: Two functional domains of CheA are shown. CheA P2 domain (purple) is connected to the P1 domain (pink) through a linker region. CheY (yellow) interacts with these two domains of CheA to allow the transfer of a phosphate from His 48 (red) of CheA to Asp 57 (blue) of CheY . Figure based on 2LP4.pdb.

CheY-CheA (P1) Binding Interactions

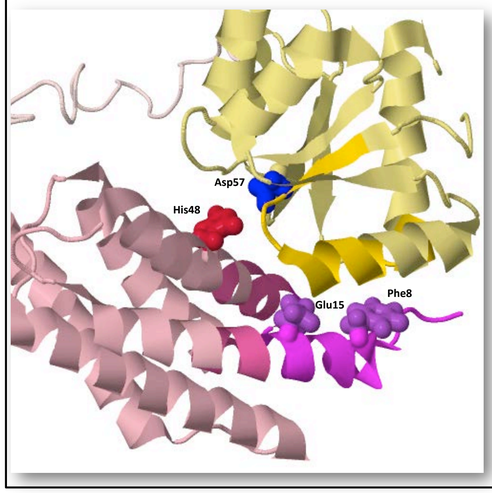

Figure 4: His48 (red) is brought in range to Asp57 (blue) through specific interactions between P1 of CheA and CheY. Residues on P1 of CheA involved in this interaction are highlighted in pink. Phe8 and Glu15 (purple) contribute largely to the association of P1 and CheY. CheY residues involved are highlighted in gold. Figure based on 2LP4.pdb.

CheY-CheA (P2) Binding Interactions

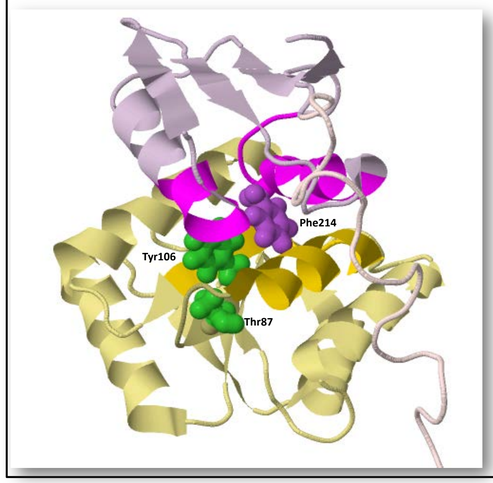

Figure 5: Phe214 of CheA (purple) increases the binding affinity of P2 to CheY one thousand fold. Tyr106 (green), exposed to the solvent, in the inactive conformation of CheY, interacts with Phe214. Other P2 residues contributing to binding affinity of CheA to CheY are highlighted in pink on CheA and yellow on CheY, including Thr87 (green). Figure based on 2LP4.pdb.

Binding Interactions Lead to Catalysis

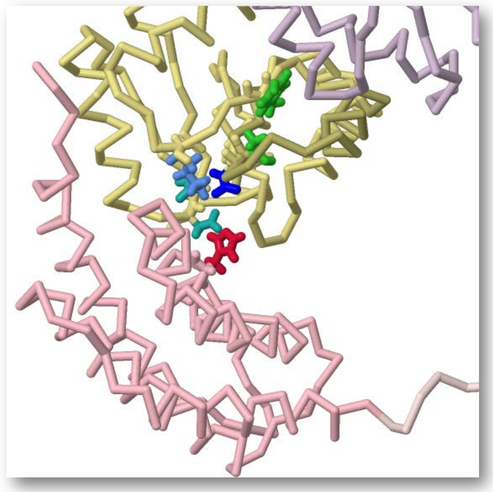

Figure 6: Structure of CheY docked to P1 and P2 domains of CheA. A phosphate is transferred from His48 (red) of CheA to Asp57 (blue) of CheY. CheY requires magnesium for catalysis; magnesium is coordinated directly by the carboxyl groups of Asp57 and Asp13 (sea foam green). Asp12 (sea foam green) coordinates the magnesium ion through a water molecule. Lys109 (light blue) orients unphosphorylated Asp57 through a salt bridge with the carboxyl group. Upon phosphorylation of Asp57, the salt bridge is broken, and Lys109 reforms a new salt bridge with the carboxyl group of Asp12. Phosphorylation of CheY causes a shift in equilibrium where Tyr106 (green) is buried in CheY and Thr87 (green) of CheY is pointed toward the active site. These shifts cause drastic changes in the β4-α4 loop that promote binding to FliM.

Summary

- P2 domain of CheA increases local concentration of CheY in the vicinity of P1.
- P1 domain of CheA has a low, but specific affinity for CheY that orients the two proteins for the phosphotransfer.
- Transfer of phosphate to the Asp57 stabilizes the active conformation of CheY by reorienting the switch residues.

References

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PDB File: 2LP4

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