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Crystal structure of pentapeptide-independent chemotaxis receptor methyltransferase (CheR) reveals idiosyncratic structural determinants for receptor recognition

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ABSTRACT

Chemotactic methyltransferase, CheR catalyse methylation of specific glutamate residues in the cytoplasmic domain of methyl-accepting chemotactic protein receptors (MCPRs). The methylation of MCPRs is essential for the chemical sensing and chemotactic bacterial mobility towards favorable chemicals or away from unfavorable ones. In this study, crystal structure of *B. subtilis* CheR (*Bs*CheR) in complex with S-adenosyl-L-homocysteine (SAH) has been determined to 1.8 Å resolution. This is the first report of crystal structure belonging to the pentapeptide-independent CheR (PICheR) class. Till date, only one crystal structure of CheR from *S. typhimurium* (*St*CheR) belonging to pentapeptide-dependent CheR (PDCheR) class is available. Structural analysis of *Bs*CheR reveals a helix-X-helix motif (HXH) with Asp53 as the linker residue in the N-terminal domain. The key structural features of the PDCheR β-subdomain involved in the formation of a tight complex with the pentapeptide binding motif in MCPRs were found to be absent in the structure of *Bs*CheR. Additionally, isothermal titration calorimetry (ITC) experiments were performed to investigate *S*-adenosyl-(L)-methionine (SAM) binding affinity and K_D was determined to be 0.32 mM. The structure of *Bs*CheR reveals that mostly residues of the large C-terminal domain contribute to SAH binding, with contributions of few residues from the linker region and the N-terminal domain. Structural investigations and sequence analysis carried out in this study provide critical insights into the distinct receptor recognition mechanism of the PDCheR and PICheR methyltransferase classes.

1. Introduction

Methyltransferases (MTases) are ubiquitously present across all life forms and are involved in site-specific covalent modification of DNAs, RNAs and proteins (Zhou et al., 2007; Sesmero and Thorpe, 2015; Li et al., 2014; Potisopon et al., 2014; Tomar et al., 2011; Niu et al., 2013; Zhang and Cheng, 2003; Cheng et al., 2011). Protein MTases (PMTs) are classified according to the residue methylated, as protein lysine MTases (PKMT), protein arginine MTase (PRMT) etc. (Haag et al., 2015; Bachand, 2007; Kaniskan et al., 2015). Small molecules such as catechol and glycine, and elements such as arsenic are also methylated by various MTases (Vidgren et al., 1994; Fu et al., 1996; Thomas et al., 2007; Ajees and Rosen, 2015). Thus, MTases strikingly play diverse roles in various biological processes such as cell signalling (Djordjevic and Stock, 1998; Batra et al., 2015; Benoit et al., 2013), epigenetic gene regulation (Tsang and Cheng, 2011; de Narvajas et al., 2013), regulation of protein-protein interaction (Dong et al., 2004; Pahlich et al., 2008), protein-DNA interaction (Ryazanova et al., 2011; Reinnisch et al., 1995), virus replication (Potisopon et al., 2014; Dong et al., 2010; Zhao et al., 2015), cancer genetics (Tsang and Cheng, 2011; Copeland, 2012, 2013) etc. As MTases play a central role in various biological processes and are site-specific, these enzymes are potential drug target for cancer (Copeland, 2012; Ghoshal and Bai, 2007), viruses (Tomar et al., 2011; Lim et al., 2015) and bacterial pathogens (Ajees and Rosen, 2015) etc.

MTases fall into five main classes, Class I–V, based on their structural features (Schubert et al., 2003). The majority of *S*-adenosyl-(L)-methionine (SAM)-dependent MTases (SAM-MTases) belong to the Class I that contains a Rossmann-fold, having seven-stranded relatively planar β-sheet sandwiched between three alpha

Abbreviations: MCPRs, methyl-accepting chemotactic protein receptors; SAH, S-adenosyl-L-homocysteine; PICheR, pentapeptide-independent CheR; PDCheR, pentapeptide-dependent CheR.

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helices on each side (Gana et al., 2013). The cofactor SAM is a classical methyl donor and plays a pivotal role in various biological reactions. The transfer of methyl group from SAM to a specific acceptor ranging from small molecules to DNAs, RNAs and proteins is catalysed by SAM-MTases. In SAM-MTases, the basic topology of central β-sheet is 3214576, with the seventh strand inserted antiparallel to the rest of the strands (Gana et al., 2013).

PMTs differ significantly in terms of their specific substrates and have been classified mainly into two broad subclasses on the basis of target residue modified, as PKMT and PRMT (Richon et al., 2011). The literature of PMTs is rich in the structures of PMTs that methylate basic residues including PRMT, PKMT, and protein histidine MTase (PHMT) (Zhang et al., 2000; Jeong et al., 2014; Xiao et al., 2003). PMTs that catalyse the methylation of acidic residues Glu and Asp at the specific site on proteins are present in both the prokaryotes as well as in the eukaryotes (Djordjevic and Stock, 1998; Griffith et al., 2001; Djordjevic and Stock, 1997; DeVry and Clarke, 1999; Sprung et al., 2008; Skinner et al., 2000; Fang et al., 2010). However, till date only one structure has been determined for protein-O-glutamate MTase (PEMT) from *S. typhimurium*, and it is the chemotactic MTase, CheR that methylates L-glutamate residue of MCPRs (Djordjevic and Stock, 1998, 1997).

Crystal structure of CheR is only available for the gram-negative bacteria *S. typhimurium* (*St*CheR) and it belongs to PDCheR class. Crystal structure of *St*CheR has been previously determined in complex with SAH (PDB ID: 1AF7) and also in complex with both SAH and the receptor pentapeptide, NWETF (PDB ID: 1BC5) (Djordjevic and Stock, 1998, 1997). *St*CheR structure consists of two domains connected by a linker. The catalytic C-terminal MTase domain of *St*CheR conforms to the basic Rossmann-type topology. This large domain contributes most of the residues for SAH-binding (Djordjevic and Stock, 1997). An extended linker connects two α-helices, one in each domain. The α-helical N-terminal domain is believed to interact with the negatively charged methylation site on the receptor, which is distinct from the receptor's pentapeptide motif (Djordjevic and Stock, 1997; Shiomi et al., 2002). In addition, the linker and the N-terminal domain also contribute residues for the cofactor binding. A small β-subdomain of *St*CheR appended to the C-terminal domain functions in the recognition of C-terminal pentapeptide tail of the receptor and recruits MTase to the receptor (PDB ID: 1BC5) (Djordjevic and Stock, 1998). However, the recruitment of CheR to the chemotactic receptors for its methylation through the C-terminal pentapeptide motif of MCPRs is a less common mechanism (Perez et al., 2004). In previous studies, the β-subdomains of CheR methytransferase have been classified into three groups. Group I includes the β-subdomains from the organisms that contain one CheR and $\geqslant$1MCP with the putative pentapeptide sequence; Group II includes the β-subdomains from the organisms that contain $\geqslant$2CheR and $\geqslant$1MCP with the putative pentapeptide sequence; and Group III includes the β-subdomains from the organisms that contain $\geqslant$1CheR and only MCPs without the putative pentapeptide sequencez (Perez and Stock, 2007). The β-subdomains of *S. typhimurium* (*St*CheR) and *B. subtilis* (*Bs*CheR) belongs to Group I and Group III β-subdomains, respectively. Three highly conserved Glycine residues of PDCheR that are accountable for pentapeptide binding are embedded within the β-subdomain β-loop (Perez and Stock, 2007). However, in PICheR including *Bs*CheR these Glycine residues are not conserved or are absent (Batra et al., 2015; Perez and Stock, 2007).

Chemotactic MTase, CheR is divided into two classes: PDCheR and PICheR on the basis of receptor recognition mechanism (Perez et al., 2004). Majority of bacteria employ CheR which falls into the PICheR class (Perez et al., 2004). However, so far, no crystal structure of CheR MTase from the PICheR class has been reported. This study reports the first crystal structure of PICheR MTase class in complex with SAH from *B. subtilis*. Structural comparison of *Bs*CheR and *St*CheR was done to investigate the structural differences in the PDCheR and PICheR. Structural analysis revealed that the C-terminal catalytic domain that contains the SAM binding Rossmann-fold is structurally similar. However, as expected the β-subdomain appended to the C-terminal domain is found to be dissimilar. The N-terminal domain sequence as well as the spatial orientation is distinct from the PDCheR MTase. This study emphasizes and categorizes the key structural features of PICheR that might be responsible for the distinct mechanism for the recruitment of CheR to MCPRs.

2. Materials and methods

2.1. Expression and purification

CheR was expressed and purified as previously described (Batra et al., 2015). Briefly, the recombinant plasmid containing *Bs*CheR gene was transformed into *E. coli* strain *Rosetta* (*DE3*) cells and plated on LB agar plate containing 50 μg/ml kanamycin and 35 μg/ml chloramphenicol to produce the N-terminal His-tagged *Bs*CheR protein. The transformed single colony was used to inoculate LB broth supplemented with 50 μg/ml kanamycin and 35 μg/ml chloramphenicol and allowed to grow overnight. The overnight grown culture of *Rosetta* (*DE3*) cells harbouring recombinant plasmid for CheR expression was used to inoculate 1L growth media. The large scale culture was allowed to grow at 37 °C until the culture reached an optical density of 0.6 at 600 nm (OD_{600}). The culture was induced with 0.8 mM isopropyl-β-thiogalactopyranoside (IPTG) at this point and the induction was allowed to continue for 16 h at 18 °C. The cells were harvested and checked for expression on sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE). Cell pellet was resuspended in 30 ml of binding buffer containing 50 mM Tris HCl (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$, 5% glycerol and 1 mM β-mercaptoethanol and disrupted by sonication on ice for 5 min with an on and off pulse of 30 s (QSonica, U.S.A). The insoluble cellular material was removed by centrifugation at 14,000*g* for 45 min at 4 °C. CheR was separated from other bacterial proteins using Nickel (Ni)-affinity chromatography by running a gradient of imidazole (30–250 mM) present in the binding buffer. The pure fractions containing *Bs*CheR were pooled and TEV protease was added to the pooled fraction in 1:25 ratio (TEV: *Bs*CheR). The protein was subsequently dialyzed against buffer containing 50 mM Tris (pH 7.5), 20 mM NaCl and 3 mM EDTA for 3 h and then against the same buffer without EDTA overnight at 4 °C. Sample was again loaded onto Ni-affinity chromatography column. Flow-through containing *Bs*CheR with cleaved His-tag was collected. After each step of purification, the fractions were analyzed on 12% SDS-PAGE and stained with Coomassie Brilliant Blue dye. Size-exclusion chromatography was also performed. Purified protein was concentrated to ~7 mg/ml using 10 kDa cut-off Amicon Ultra-15 concentrator (Millipore) at 4 °C. The concentration of purified protein was estimated by UV–vis spectroscopy at 280 nm using the extinction coefficient of ~35230 $cm^{-1}M^{-1}$ for *Bs*CheR.

2.2. Isothermal titration calorimetry

Isothermal Titration Calorimetry (ITC) experiments were conducted using MicroCal iTC200 (GE Healthcare) to determine the thermodynamic parameters of SAM binding to the purified *Bs*CheR protein. SAM used in the ITC titration against *Bs*CheR protein was purchased from Sigma Aldrich (Cat No. A7007). The titrations were performed at 25 °C in buffer containing 50 mM Tris (pH 7.5) and 20 mM NaCl. 2 mM SAM (Syringe) dissolved in the same buffer

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