Crystal structure of CheA, the histidine kinase central to bacterial chemotaxis.

Alexandrine M. Bilwes, Lisa A. Alex, Brian R. Crane, and Melvin I. Simon.
Department of Biology, California Institute of Technology, Pasadena, California 91125.

INTRODUCTION

CheA, a representative histidine kinase, is a cytoplasmic central component of signal transduction pathways that allow bacteria to sense and respond to their environment [1, 2]. Most bacteria are able to swim by rotating their helical flagella. When the flagellar bundle rotates counterclockwise the bacterium swims smoothly. Switching to clockwise rotation results in a reorientation (tumbling) of the bacterium. The signaling circuit that controls the switching consists of transmembrane chemoreceptors that through an adaptor protein CheW regulate the histidine auto-phosphorylation of the multi-domain CheA protein. CheY obtains the phosphate from CheA and becomes the signal to change the direction of flagellar rotation.

RESULTS AND DISCUSSION

We recombinantly expressed *Thermotoga maritima* CheAΔ289 [3] (residues 290-671) which lacks the two amino terminal domains, P1 and P2. CheAΔ289 phosphorylates the fragment composed of *T. maritima* P1 and P2 *in vitro* (data not shown). In solution, CheA Δ289 forms a complex with recombinant *T. maritima* CheW and the two proteins copurify. Thus, CheAΔ289 is enzymatically active and retains substrate specificity and binding to CheW.

We determined the crystal structure of CheAΔ289 at 2.6 Å resolution by combining experimental phases (Table 1 and 2) from multiple isomorphous replacement (MIR) with phases from multiwavelength anomalous diffraction (MAD). Synchrotron radiation was essential to this project as our crystals did not diffract beyond 3.6 Å using a rotating anode as a source of X-rays.

Dimeric recombinant wild type CheAΔ289 and mercury-modified mutants Q545C and E387C produced crystals suitable for structure determination (Table 1). Crystals of mercury-modified Q545C diffracted better than wild type CheAΔ289 and were highly isomorphous with unmodified Q545C (Table 1). MAD data collected at ALS beamline 5.0.2 on mercury-modified and seleno-methionine substituted CheAΔ289 was essential for structure determination. Anomalous diffraction from the seleno-methionine containing protein crystals was used to locate 20 methionines in the electron-density map and aided the definition of the non-crystallographic symmetry and the tracing of the model. Cycles of model-weighted phase combination, manual rebuilding with interactive graphics, and reciprocal space refinement produced the final structure. Our 2.6 Å resolution refined model of mercury modified CheAΔ289 Q545C (R-factor of 21.3%; R-free 28.5 %) includes a non-symmetric dimer of residues 293-671, 2 mercury atoms, 221 water molecules, and displays reasonable geometry.

Table 1. Native and derivative data statistics.

Data set	Resolution* (Å)	R-sym # (%)	I/σ(I)&	Complete-ness (%)	Reflections Unique	Reflections Observed	Wave length (Å)	sites
545Hg	2.6(2.7-2.6)	6.8(34.4)	21.2(3.2)	92.6(79.5)	33278	93352	1.08	2
545	2.5(2.6-2.5)	3.9(28.9)	16.9(2.9)	78.5(67.5)	31655	57757	1.08	2
387Hg	3.2(3.3-3.2)	5.4(26.9)	17.2(4.8)	84.4(78.0)	16572	31820	1.08	2
Hg3	2.7(2.8-2.7)	6.1(31.6)	25.4(7.6)	93.8(97.1)	31863	170975	0.946	2
Seleno 545	2.5(2.6-2.5)	6.2(29.6)	37.2(8.5)	98.2(98.9)	40131	196467	0.979	20
Hg4 peak	2.7(2.8-2.7)	3.9(30.7)	19.4(5.7)	93.2(95.1)	29593	76121	1.001	2
Hg4 inflection	2.6(2.7-2.6)	5.8(28.3)	18.5(5.0)	92.5(95.7)	32719	80860	0.999	2
Hg4 high remote	2.7(2.8-2.7)	5.5(29.1)	17.9(5.5)	92.8(95.0)	29847	73990	0.946	2

545Hg, Hg3, Hg4 represent different data sets of CheAΔ289 Q545C soaked in $HgCl_2$; 387Hg represents CheAΔ289 E387C co-crystallized with EMTS; and Seleno 545 represents selenomethionine CheA Δ289 Q545C. The crystal Hg4 was used for the collection of MAD data.

*Highest resolution of data set followed by resolution range in highest bin for compiling statistics.

#Rsym=ΣΣj | Ij-⟨I⟩|/Σ⟨I⟩ , I=intensity, & I/σ(I) =signal-to-noise. The 3 first data sets were collected at SSRL beamline 7-1 on a 30 cm Mar Research Image plate detector; the others were collected at ALS beamline 5.0.2 on a CCD 2x2 quantum detector.

Table 2. MIR & MAD phasing statistics.

	Resolution (Å)	Phasing power iso*	Phasing power ano**	R*iso* (%)	R*cen* (%)	R*K* (%)	Anomalous ratio	Dispersive Ratio (vs inflection)	Figure of merit (2.8Å)
545	2.8	2.18		15.8	52.2				
387Hg	3.5	1.03	0.87	39.2	69.3	50.7	4.6		MIRAS
Hg3	2.8		2.30	15.0		16.6	4.8		0.56
545Hg anomalous	3.5		1.59			17.2	4.3		
Hg4 inflection	2.8						4.1		MAD 0.47
Hg4 peak	2.8						4.9	9.8	
Hg4 high remote	2.8						5.1	10.6	
Combined	2.8								0.67

MIR statistics are calculated with 545Hg as the reference data set.

*Phasing power iso = ⟨|FH|⟩/⟨*E*⟩ where |FH| is the calculated heavy atom structure factor amplitude and *E* is the lack of closure error. **Phasing power ano = ⟨2|FH''|⟩/⟨*E*⟩ where |FH''| is the anomalous correction component amplitude. R*iso* = ΣΣj||Fj|-⟨|F|⟩|/Σ⟨|F|⟩. R*cen* = Σ||FPH|-|FP| -|FH||/Σ||FPH|-|FP|| for centric reflections of isomorphous derivatives.

R*K* = Σ||FPH^+|-|FPHcalc$^+$|| + ||FPH^-|-|FPHcalc$^-$|| / |FPH^+| + |FPH^-| for anomalous scattering.

Anomalous ratio =rms||$F^{+|}$|-| F^- ||/rms F. Dispersive ratio = rms||$F_{\lambda1}$|-|$F_{\lambda2}$||/rms F , rms=root mean square.

The crystal structure of CheA (residues 290-671) histidine kinase reveals a dimer (Figure 1) where the functions of dimerization, ATP-binding, and regulation are segregated into domains. The kinase domain is unlike Ser/Thr/Tyr kinases but resembles three ATPases: GyraseB [4], MutL [5] and Hsp90 [6]. Structural analogies within this superfamily suggest that the P1 domain of CheA provides the nucleophilic histidine and activating glutamate for phosphotransfer. CheA (dimerization and kinase domains) is also topologically similar to the dimeric phosphotransferase Spo0B [7]. However, the kinase-like domain in Spo0B is much shorter than the kinase domain in CheA and lacks all of the residues involved in binding ATP. The topological similarity between CheA and Spo0B shows that signal transduction circuits in bacteria have derived different functions from similar structural modules.

The CheA regulatory domain, which binds the homologous receptor-coupling protein CheW, topologically resembles two SH3 domains and provides different protein recognition surfaces at each end. The CheA dimerization domain forms a central 4-helix bundle about which the kinase and regulatory domains pivot on conserved hinges to modulate trans-phosphorylation. Different subunit conformations suggest that relative domain motions link receptor response to kinase activity.

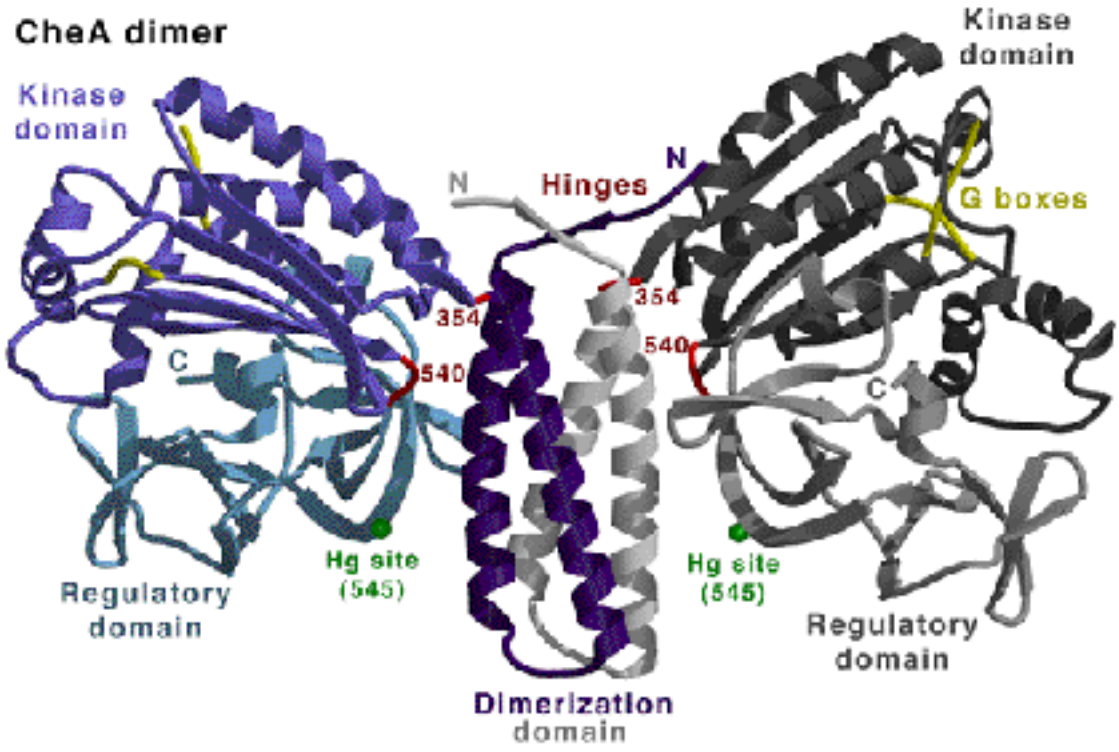

Figure 1. Overall structure of the CheA_289 dimer
The 2 subunits (blue for MOL1, gray for MOL2) of the CheA dimer (shown as ribbon diagram) each contain separate dimerization, kinase, and regulatory domains. The dimer associates by a central 4-helix bundle and places the 2 ATP binding sites (located by the G-boxes) 90 Å away from each other.

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Principal investigator: Melvin I. Simon, Division of Biology, California Institute of Technology. E-mail: simonm@cco.caltech.edu. Telephone: (626) 395-3944.