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Crystallization and preliminary X-ray crystallographic analysis of *Thermotoga maritima* CheA P3-P4-P5 domains in complex with CheW

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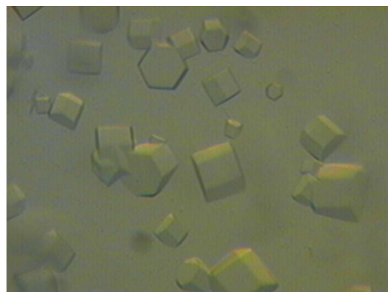
The CheA–CheW complex plays a key role in bacterial chemotaxis signal transduction by initiating phosphotransfer to response regulators *via* coupling to the chemoreceptors. CheA (P3-P4-P5 domains) and CheW from *Thermotoga maritima* were overexpressed in *Escherichia coli* and crystallized as a complex at 298 K using ammonium dihydrogen phosphate as a precipitant. X-ray diffraction data were collected to ~8 Å resolution at 100 K using synchrotron radiation. The crystal belonged to space group $I222$ or $I2_12_12_1$, with unit-cell parameters a = 184.2, b = 286.4, c = 327.7 Å. The asymmetric unit may contain six to ten CheA–CheW molecules.



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1. Introduction

Chemotaxis enables bacteria to swim towards an attractant (nutrient) or away from a repellent with remarkable sensitivity, gain, dynamic range and feedback control (Wadhams & Armitage, 2004; Sourjik, 2004; Parkinson *et al.*, 2005). Signal transduction begins with the membrane-spanning chemoreceptors (also known as methyl-accepting chemotaxis proteins or MCPs) sensing the environmental cue and triggering the histidine kinase CheA, which has five domains (P1–P5), to initiate a phosphorelay necessary to change the flagellar rotation. The cytoplasmic region of the chemoreceptor distal to the membrane interacts with CheA through a coupling protein CheW, and CheA binds tightly to CheW *via* the P5 regulatory domain (P5) of CheA (Park *et al.*, 2006; Bhatnagar *et al.*, 2010; Miller *et al.*, 2006). A large surface area (~1050 Å^2 per domain) is buried between CheA P5 and CheW, and is mostly mediated by conserved hydrophobic residues on intertwined five-stranded β-barrels (Park *et al.*, 2006).

A pulsed electron spin resonance (ESR) based solution structure of CheA P3-P4-P5 domains[1] (P3-P4-P5; P3 is the CheA dimerization domain and P4 is the CheA kinase domain) and CheW suggested a model for the interaction of CheA and CheW (Park *et al.*, 2006). The model suggested that interaction between CheW and CheA P3 may be important in the signalling event, supporting the data that the affinity of CheW and CheA increases more than tenfold when P3 is fused to P4-P5 of CheA (K_d = 10 *versus* 130 nM) and that the CheA dimer subunit exchange mediated by P3 is stabilized in the presence of CheW (Park *et al.*, 2004). Despite the fact that the ESR model is simultaneously supported by the X-ray crystal structures of CheA P4-P5 and CheW, the detailed molecular interaction between CheW and P3 remains elusive.

Since chemoreceptor clustering at the bacterial pole is essential for the normal function of chemotaxis signalling, the modes of CheA and CheW association in the cytoplasm must coincide with co-localization of the chemoreceptors. Clustering of CheA mediated by P5 has been proposed from the identical crystal packing of P5 in two different crystal forms (Park *et al.*, 2006; Bilwes *et al.*, 1999). Hence, a study of the crystal structure of the complex of CheA P3-P4-P5 and CheW may further confirm the oligomeric interaction modes of CheA and CheW relevant to mediating the chemotaxis signal. As a first step,

[1] The other domains of CheA are the P1 phosphotransfer domain and the P2 response regulator binding domain.

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we report the overexpression, crystallization and preliminary X-ray crystallographic data of the complex of *Thermotoga maritima* CheA Δ289 (P3-P4-P5) and CheW.

2. Experimental

2.1. Protein overexpression and purification

The genes encoding *T. maritima* CheA Δ289 (P3-P4-P5 domains; CheA residues 290–671) and full-length *T. maritima* CheW (1–151) were PCR-cloned into the vector pET28 (Novagen) using *T. maritima* genomic DNA (ATCC). The proteins with N-terminal His_6 tags were expressed in *Escherichia coli* strain BL21 (DE3) (Stratagene) with kanamycin selection (25 µg ml^{-1}). The transformed cells were grown at 310 K in 2 l Terrific Broth medium to an OD_{600} of 0.6 using a conventional shaker. Recombinant protein expression was induced with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) and the cells were grown for a further 16 h at 298 K. The cells were harvested by centrifugation at 4500*g* for 10 min at 277 K and were resuspended in ice-cold lysis buffer (20 m*M* Tris pH 7.5, 500 m*M* NaCl, 5 m*M* imidazole) prior to homogenization by sonication. The cell lysates were centrifuged at 70 000*g* for 30 min at 277 K and the supernatants were loaded onto nickel–NTA-loaded columns. The columns were washed with wash buffer (20 m*M* Tris pH 7.5, 500 m*M* NaCl, 20 m*M* imidazole) and the recombinant proteins were eluted with elution buffer (20 m*M* Tris pH 7.5, 500 m*M* NaCl, 200 m*M* imidazole). The His_6 tag was removed by treating the eluted protein with human thrombin (Roche) for 16 h at 277 K. The purified CheA Δ289 and CheW proteins were concentrated by centrifugation using YM-30 Centriprep (Amicon Millipore), mixed in an approximately 1:3 (CheA Δ289:CheW) ratio and further purified using a Superdex 200 26/60 sizing column (GE Healthcare) equilibrated with gel-filtration buffer (50 m*M* Tris pH 7.5, 150 m*M* NaCl). The elution profile gave two major peaks, with the higher molecular-weight peak containing the CheA Δ289–CheW complex and the lower molecular-weight peak containing excess CheW. SDS–PAGE analysis confirmed that the higher molecular-weight peak contained the complex of CheA and CheW. The elution fractions corresponding to the complex were concentrated to ∼50 mg ml^{-1} by centrifugation using YM-30 Centriprep. The concentration of the complex was estimated by absorption at λ = 280 nm employing the calculated molar extinction coefficient of 24 180 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

2.2. Crystallization and X-ray data collection

Initial conditions for the growth of *T. maritima* CheA Δ289–CheW crystals were found using commercial screening solutions (Hampton Research). Crystallization screening was set up in 24-well Linbro plates using the hanging-drop vapour-diffusion method at 298 K. Each drop (2 µl) was prepared by mixing equal volumes of purified protein solution (∼50 mg ml^{-1}) and reservoir solution. Initial clusters of needle-shaped crystals were obtained using a reservoir solution consisting of 1.0 *M* $NH_4H_2PO_4$. The condition was optimized to grow crystals in a single form by using additive and detergent screens. Final diffraction-quality crystals were obtained using a reservoir solution consisting of 0.5–1.0 *M* $NH_4H_2PO_4$, 5–10 m*M* *n*-octyl-β-D-glucopyranoside (BOG). SDS–PAGE analysis confirmed that the crystals contained both CheA and CheW. Diffraction data were collected under a 100 K nitrogen stream using an ADSC Quantum Q315 CCD detector on the NE-CAT beamlines at APS. Data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystal belonged to space group *I*222 (or $I2_12_12_1$) and may contain between six and ten molecules of CheA Δ289–CheW per asymmetric unit as indicated by Matthews coefficient prediction (Matthews, 1968).

3. Results

Recombinant *T. maritima* CheA Δ289 and *T. maritima* CheW with N-terminal His_6 tags were overexpressed in *E. coli* as soluble proteins, with an overall yield of ∼100 mg purified protein per litre of culture. The two proteins eluted as a complex from a size-exclusion column. Diffracting crystals were obtained using a reservoir solution consisting of 0.5–1.0 *M* $NH_4H_2PO_4$, 5–10 m*M* BOG. Crystals grew to approximate dimensions of 50 × 50 × 20 µm within one week and contained both CheA and CheW, as confirmed by SDS–PAGE analysis (Fig. 1).

For X-ray diffraction experiments, a crystal from a hanging drop was transferred into a nitrogen cryostream after a quick soak in mother-liquor solution containing 5–15%(*v*/*v*) glycerol, which acted as a cryoprotectant. The diffraction images showed isotropic diffraction, but only to a low resolution.

A complete set of ∼8 Å resolution diffraction data was collected using synchrotron radiation. A total of 7379 unique reflections were obtained with an R_{merge} (on intensity) of 23.2%. The crystal belonged to the body-centred orthorhombic space group *I*222 (or $I2_12_12_1$). The unit-cell parameters were *a* = 184.2, *b* = 286.4, *c* = 327.7 Å. Since

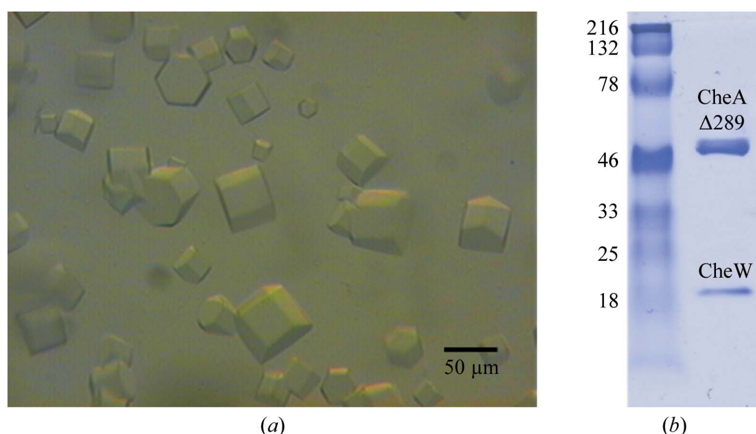

Figure 1
(*a*) Crystals of *T. maritima* CheA Δ289–CheW, the complex of the histidine kinase and chemoreceptor coupling protein in bacterial chemotaxis signalling. (*b*) SDS–PAGE analysis of CheA Δ289–CheW crystals. Molecular-weight markers are labelled in kDa.

Table 1
Data-collection statistics.

Values in parentheses are for the outer shell.

X-ray wavelength (Å)	0.9783
Temperature (K)	100
Resolution range (Å)	50–8.50 (8.80–8.50)
Space group	$I222$ or $I2_12_12_1$
Unit-cell parameters (Å)	a = 184.2, b = 286.4, c = 327.7
Unique reflections	7379 (656)
Multiplicity	5.1 (4.4)
Completeness (%)	94.5 (86.1)
Mean $I/\sigma(I)$	7.5 (2.5)
$R_{\rm merge}$† (%)	23.2 (52.8)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith measurement of reflection hkl and $\langle I(hkl)\rangle$ is the mean value of $I_i(hkl)$ for all i measurements.

CheA dimerizes *via* the P3 domain, the presence of six to ten molecules of a (CheA Δ289–CheW)$_2$ complex is plausible in the crystal asymmetric unit according to the Matthews coefficient. (For six to ten molecules in the asymmetric unit, the Matthews coefficient ranges between 3.0 and 1.8 Å^3 Da^{-1}, with a solvent content in the range 59–32%. However, a solvent content of 30–40% is unlikely owing to the relatively low diffraction power of the crystal at the synchrotron.) The statistics for the data are summarized in Table 1. We attempted to search for a molecular-replacement solution using previously reported structures of CheA Δ289 (Bilwes *et al.*, 1999) and CheW (Park *et al.*, 2006; Griswold *et al.*, 2002) but failed, probably resulting from the limited resolution quality of the data.

We will continue our efforts to determine the structure of the complex by improving the crystals for higher resolution diffraction. We intend to utilize systematic crystallization strategies to improve the diffraction. The addition of various detergents, organic additives, and heavy-metal salts of mercury, lead or cobalt to the crystallization condition and the use of selenomethionine-substituted protein have been shown to improve diffraction quality. Dehydrating the crystals to reduce the solvent content, annealing the crystals and cross-linking the crystals with glutaraldehyde are other methods for improving diffraction (Heras *et al.*, 2003; Harp *et al.*, 1998; Lusty, 1999). We are also performing crystallization from D_2O-based buffers, which can enhance the quality of diffraction by increasing the hydrophobic effect in the protein complex. Use of a microfocus beam is also under consideration.

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