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Crystallization and preliminary X-ray crystallographic analysis of CheW from *Thermotoga maritima*: a coupling protein of CheA and the chemotaxis receptor

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The CheW protein plays a key role in bacterial chemotaxis signal transduction by coupling CheA to chemotaxis receptors. CheW from *Thermotoga maritima* has been overexpressed in *Escherichia coli* and crystallized at 298 K using ammonium sulfate as a salt precipitant. X-ray diffraction data have been collected to 3.10 Å resolution at 100 K using synchrotron radiation. The crystal belonged to space group $P6_3$, with unit-cell parameters $a = b = 61.265$, $c = 361.045$ Å. The asymmetric unit may contain four to six CheW molecules.

1. Introduction

Bacterial chemotaxis is a signal transduction cascade which enables bacteria to swim towards an attractant (nutrient) or away from a repellent. Ligand occupation at the periplasmic region of chemotaxis receptors (also known as chemoreceptors, methyl-accepting chemotaxis proteins or MCPs) influences the cytoplasmic histidine kinase CheA; the two proteins are joined by a coupling protein CheW. This membrane-spanning receptor–CheW–CheA complex tunes the activity of CheA and allows the bacterium to sense extracellular signals with remarkable sensitivity, gain, dynamic range and feedback control (Wadhams & Armitage, 2004; Sourjik, 2004; Parkinson *et al.*, 2005).

The cytoplasmic region of the chemotaxis receptor is a long dimeric antiparallel four-helix bundle (∼250 Å) and the site most distal to the membrane interacts with CheA through the coupling protein CheW (Park *et al.*, 2006; Bhatnagar *et al.*, 2010; Miller *et al.*, 2006). CheW shares an SH3 domain-like fold with the CheA P5 regulatory domain. Both proteins are composed of two intertwined five-stranded β-barrels (Park *et al.*, 2006; Griswold *et al.*, 2002). As seen from the complex structure of the CheA P5 domain and CheW, tight interactions between the P5 proximal β-barrel and CheW subdomain 2 bury a large amount surface area, which is mostly mediated by conserved hydrophobic residues (Park *et al.*, 2006). Dimeric CheA–CheW, as defined by crystallography and pulsed ESR, positions two CheW molecules in a cleft that is lined with residues that are important for receptor interactions and is sized to clamp one dimer of a chemoreceptor (Park *et al.*, 2006). A recent study mapping the interaction of the receptor, CheA and CheW has suggested an asymmetric mode of interaction among the partners (Bhatnagar *et al.*, 2010).

Since the clustering of chemotaxis receptors at the extracellular pole of the bacterium is essential for normal signalling function, the localization of receptors can be related to the association modes of CheA and CheW in the cytoplasm. As clustering of CheA mediated by the P5 domain has previously been proposed (Park *et al.*, 2006), CheW may also form molecular clusters by self-interaction. Although the *Thermotoga maritima* CheW structure has been reported from solution NMR and from a crystal of a complex with CheA, further CheW oligomeric states may be observed in other crystal-packing environments. For example, the previously reported crystal structure of *Thermoanaerobacter tengcongensis* CheW contained two CheW molecules that form a dimer by twofold noncrystallographic symmetry (Yao *et al.*, 2007) despite CheW behaving as a monomer in solution (Yao *et al.*, 2007; Park *et al.*, 2006; Griswold *et al.*, 2002). Since the clustering state of CheW and its relevance in mediating the

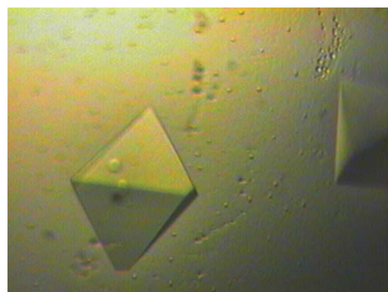

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Table 1
Data-collection statistics.

X-ray wavelength (Å)	0.936
Temperature (K)	100
Resolution range (Å)	30–3.10 (3.21–3.10)
Space group	$P6_3$
Unit-cell parameters (Å)	$a = b = 61.265$, $c = 361.045$
Unique reflections	134351 (10761)
Multiplicity	2.8 (2.5)
Completeness (%)	86.9 (89.2)
Mean $I/\sigma(I)$	18 (4.7)
R_{merge}† (%)	7.5 (36.7)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, 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.

receptor–CheW–CheA complex can be hypothesized from the crystal structure of *T. maritima* CheW, structure determination and comparison of the oligomeric interaction modes with those of *T. tengcongensis* CheW merits investigation. As a first step, we here report the overexpression, crystallization and preliminary X-ray crystallographic data of *T. maritima* CheW.

2. Experimental

2.1. Protein overexpression and purification

The gene encoding full-length CheW (residues 1–151) was PCR-cloned into the vector pET28a (Novagen) using *T. maritima* genomic DNA (ATCC). The protein was expressed with N-terminal His_6 tags in *Escherichia coli* strain BL21 (DE3) (Stratagene) using 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 at 298 K for a further 16 h. The cell pellets were harvested using 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* sodium chloride and 5 m*M* imidazole) prior to homogenization by sonication. The cell lysates were centrifuged at 70 000*g* for 30 min at 277 K. The supernatant was loaded onto nickel–NTA columns and washed with wash buffer (20 m*M* Tris pH 7.5, 500 m*M* sodium chloride and 20 m*M* imidazole) and the recombinant protein was eluted with elution buffer (20 m*M* Tris pH 7.5, 500 m*M* sodium chloride and 200 m*M* imidazole). The His_6 tag was removed by adding thrombin (Roche) to the eluate for 16 h at 277 K. The protein was further purified using a Superdex 200 sizing column (GE Healthcare) equilibrated with gel-filtration buffer (50 m*M* Tris pH 7.5 and 150 m*M* NaCl) and was concentrated to ~110 mg ml^{-1} by centrifugation using a YM-10 Centriprep (Amicon Millipore). Protein concentrations were estimated from the absorption at λ = 280 nm employing the calculated molar extinction coefficient of 1280 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

2.2. Crystallization and X-ray data collection

The initial conditions for growing *T. maritima* CheW crystals were found using commercial screening solutions (Hampton Research). Initial crystals grew by vapour diffusion against a reservoir consisting of 1.6–2.4 *M* lithium sulfate and 0.1 *M* HEPES pH 7.5. The diffraction quality of the initial crystals was improved by screening with different pH buffers and with organic additives such as dioxane or glycerol. Substitution of lithium sulfate by ammonium sulfate also improved the diffraction quality. The final CheW crystals used in the diffraction experiment grew by vapour diffusion against a reservoir consisting of 2.1–2.6 *M* ammonium sulfate, 0.1 *M* MES pH 6.5 and 5–10% glycerol. Diffraction data were collected in a 100 K nitrogen stream on CHESS beamline A1a using a CCD detector (ADSC Quantum Q210) and were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystal belonged to space group $P6_3$ and contained between four and six molecules of CheW per asymmetric unit, as indicated by the Matthews coefficient prediction.

3. Results

Recombinant *T. maritima* CheW with an N-terminal His_6 tag was overexpressed in *E. coli* as a soluble protein with a yield of ~100 mg purified protein per litre of culture. The protein eluted as a monomer on a size-exclusion column. The best crystals were obtained using a reservoir solution consisting of 2.1–2.6 *M* ammonium sulfate, 0.1 *M* MES pH 6.5 and 5–10% glycerol. Crystals grew to approximate dimensions of 300 × 300 × 100 µm within one week.

Crystals could be transferred directly from the hanging drop to the nitrogen cryostream for X-ray diffraction experiments, as the glycerol in the mother liquor acted as a cryoprotectant. The diffraction images showed isotropic diffraction; owing to the extremely long *c* axis the crystal had to be positioned with one specific crystal axis perpendicular to the beam in order to achieve completeness in all of the resolution shells.

A set of diffraction data to 3.10 Å resolution was collected using synchrotron radiation. A total of 10 761 unique reflections had an R_{merge} (on intensity) of 7.5%. Systematic absences indicated that the crystal belonged to the hexagonal space group $P6_3$. The unit-cell parameters are $a = b = 61.265$, $c = 361.045$ Å. The presence of four to six subunits of CheW in the crystal asymmetric unit is plausible according to the Matthews coefficient (Matthews coefficient of 2.8 Å^3 Da^{-1} and solvent content of 55% for four molecules, Matthews coefficient of 2.2 Å^3 Da^{-1} and solvent content of 44% for five molecules, and Matthews coefficient of 1.8 Å^3 Da^{-1} and solvent content of 33% for six molecules). The data statistics are summarized in Table 1. We used various programs to perform molecular replacement using several CheW structures, but failed to find a correct solution. In the near future, we plan to determine the structure by direct phasing methods in conjunction with improvement of the CheW crystals to obtain higher resolution diffraction. The oligomeric state of CheW in the crystals may suggest interactions that are of relevance to the assembly of the receptor–CheW–CheA complex.

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