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Crystallization and preliminary X-ray crystallographic analysis of *Escherichia coli* CheA P3 dimerization domain

Sang Youn Park,[a]* Sang Woo Ham,[a] Keon Young Kim[a] and Brian R. Crane[b]

[a]School of Systems Biomedical Science, Soongsil University, 511 Sangdo-Dong, Dongjak-Gu, Seoul, South Korea, and [b]Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York, USA

Correspondence e-mail: psy@ssu.ac.kr

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The chemotaxis histidine kinase CheA assembles into a dimer in which the P3 dimerization domain forms a four-helix bundle by the parallel association of two α-helical hairpins from each subunit. Ligand occupancy of the chemoreceptor regulates signal transduction by controlling the autophosphorylation activity of CheA. Autophosphorylation of CheA occurs *in trans*, *i.e.* one subunit phosphorylates the other. The P3 domain of CheA from *Escherichia coli* has been overexpressed in *E. coli* and crystallized at 298 K using PEG as a precipitant. X-ray diffraction data to 2.80 Å resolution have been collected at 100 K using synchrotron radiation. The crystal belonged to space group *P*1, with unit-cell parameters $a = 59.271$, $b = 67.674$, $c = 82.815$ Å, $\alpha = 77.568$, $\beta = 86.073$, $\gamma = 64.436^\circ$. The asymmetric unit may contain up to ten dimeric units of P3 four-helix bundles.

1. Introduction

Bacterial chemotaxis describes the behaviour in which bacteria swim towards an attractant or away from a repellent. The underlying signalling mechanism involves ligand binding to the periplasmic region of chemoreceptors (also known as methyl-accepting chemotaxis proteins or MCPs), which then regulate the activity of the cytoplasmic histidine kinase CheA. Dimeric CheA initiates an intracellular phosphorelay by transferring phosphate from ATP to a specific histidine residue. Phosphate is then transferred to an aspartate residue of the response regulator CheY, which in turn binds to the flagellar motor and switches the direction of filament rotation. In general, bacterial chemotaxis achieves remarkable sensitivity, gain, dynamic range and feedback control by interpreting extracellular signals and tuning the activity of CheA (Wadhams & Armitage, 2004; Parkinson *et al.*, 2005).

Dimerization of CheA is required for intersubunit transphosphorylation (Swanson *et al.*, 1993; Ellefson *et al.*, 1997) and the crystal structure of the *Thermotoga maritima* CheA P3-P4-P5 domains defined the P3 domain as the primary unit that mediates dimerization. The four-helix bundle of the P3 domain is formed *via* the parallel association of two helical hairpins from each subunit (Fig. 1; Bilwes *et al.*, 1999). In addition to assembling CheA for transphosphorylation, the P3 domain also mediates dynamic exchange of the CheA subunits. In overcoming the large energy barrier generated by exposing the extensive hydrophobic buried interface (~2000 Å^2 per subunit) of the four-helix bundle, CheA dimers exchange subunits by rate-limiting dissociation of the P3 domain (Park *et al.*, 2004). CheA subunit dissociation in the mesophile *Escherichia coli* and the thermophile *T. maritima* is activated at the temperatures at which the respective organisms live (310 and 353 K, respectively), which further indicates that P3-domain-mediated subunit exchange has functional relevance.

Despite the differences in the P3-domain dissociation rates, the aligned P3-domain sequences that mediate the tight interaction of the four-helix bundle are well conserved in both *E. coli* and *T. maritima* (Fig. 1). Only the length of the loop connecting the two α-helices shows a considerable difference in the proteins derived from the two

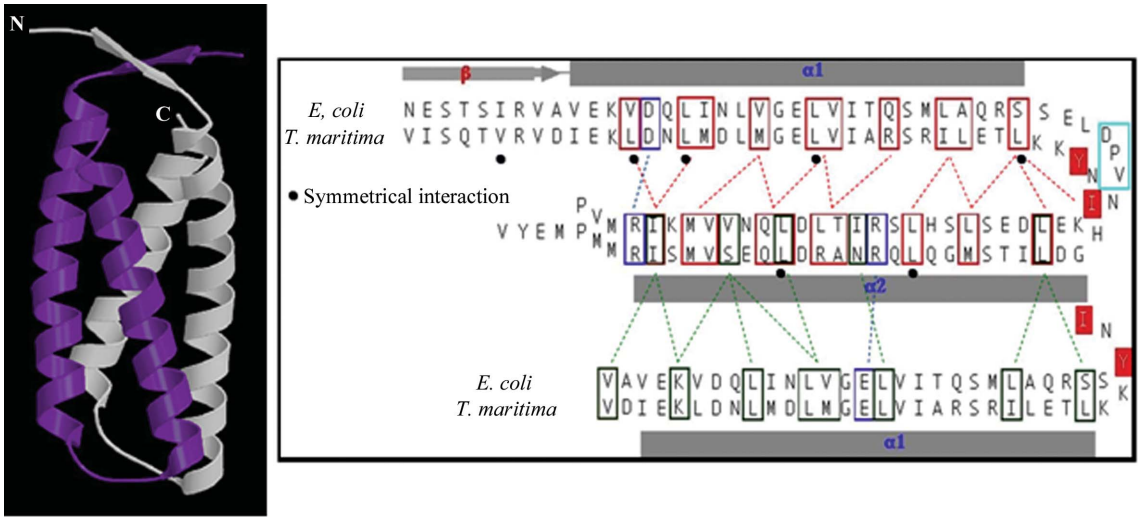

Figure 1
Structure of the *T. maritima* CheA P3 dimerization domain and sequence alignment of the *T. maritima* and *E. coli* P3 domains showing the interactions that stabilize the four-helix bundle. Hydrogen-bond-forming residues (blue) and hydrophobic interaction-mediating residues (red) within the same subunit, as well as the residues mediating hydrophobic interactions with the opposite subunit (green), are indicated by boxes around the residues. Symmetric hydrophobic interactions in which the residues interact with the same residues of the opposite subunit (black dots) and residues within the loop which participate in the formation of hydrophobic interactions in the case of *T. maritima* (red filled boxes) are shown. The three additional residues in the loop of the *E. coli* P3 domain are also indicated (sky blue box).

species. In order to correlate the difference in subunit-exchange rate with the structural differences between the two species, it is necessary to obtain a high-resolution structure of the *E. coli* CheA P3 domain. In the first step towards this structure determination, here we report the overexpression, crystallization and preliminary X-ray crystallographic data of the *E. coli* P3 dimerization domain.

2. Experimental

2.1. Protein overexpression and purification

The gene encoding the *E. coli* P3 dimerization domain (residues 279–332) was PCR-cloned into the vector pET28a (Novagen) using *E. coli* genomic DNA (ATCC). The protein was expressed with an N-terminal His_6 tag in *E. coli* strain BL21 (DE3) (Stratagene) using kanamycin selection (25 µg ml^{-1}). The transformed cells were grown at 310 K in 2 l LB 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 289 K for a further 16 h. The cell pellets were harvested using centrifugation at 4500*g* for 10 min at 277 K and resuspended in ice-cold lysis buffer (20 m*M* Tris pH 7.5, 500 m*M* sodium chloride and 5 m*M* imidazole). Cell lysates were prepared using homogenization by sonication and were centrifuged at 70 000*g* for 30 min at 277 K. The supernatants were 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 proteins were 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 human 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 in gel-filtration buffer (50 m*M* Tris pH 7.5, 150 m*M* NaCl and 2 m*M* DTT) and was concentrated to 25 mg ml^{-1} by centrifugation using YM-10 Centriprep (Amicon Millipore). The protein concentration was estimated from the absorption at a wavelength of 280 nm employing the calculated molar extinction coefficient of 2560 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

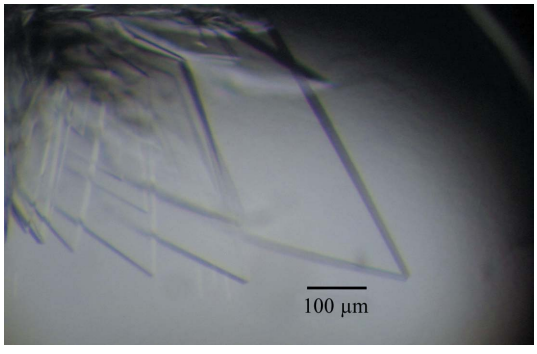

Figure 2
Crystals of the *E. coli* CheA P3 dimerization domain.

2.2. Crystallization and X-ray data collection

Conditions for growth of *E. coli* P3 dimerization domain crystals were obtained using commercial crystallization screens (Hampton Research). Crystallization screening was performed at 298 K using the hanging-drop vapour-diffusion method in 24-well Linbro plates. Protein (25 mg ml^{-1}) and well solutions were mixed in a 1:1 ratio to give a total volume of 2 µl. Initial needle-cluster crystals were obtained from well solutions consisting of 0.2 *M* ammonium sulfate, 0.1 *M* sodium cacodylate pH 6.5 and 30%(*w*/*v*) PEG 8000 or 0.2 *M* sodium acetate, 0.1 *M* Tris pH 8.5 and 30%(*w*/*v*) PEG 4000. However, modifying the PEGs and excluding the salts from the conditions improved the crystals into a single form. The final crystals used in the diffraction experiment were obtained with a reservoir solution consisting of 28–36%(*v*/*v*) PEG 3350 and 0.1 *M* Tris pH 8.5 in one week using the same method at 298 K. Crystals were soaked in mother liquor containing an additional 5%(*v*/*v*) glycerol (or ethylene glycol) for cryoprotection. Diffraction data were collected under a 100 K nitrogen stream on CHESS beamline F2a using a CCD detector (ADSC Q210). Data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystal belonged to space group *P*1 and may contain between six and ten dimeric P3-domain

Table 1
Data-collection statistics.

Values in parentheses are for the last shell.

X-ray wavelength (Å)	0.990
Temperature (K)	100
Resolution range (Å)	50–2.80 (2.90–2.80)
Space group	*P*1
Unit-cell parameters (Å, °)	a = 59.271, b = 67.674, c = 82.815, α = 77.568, β = 86.073, γ = 64.436
Total reflections/unique reflections	132599/28102
Multiplicity	1.8 (1.6)
Completeness (%)	94.5 (85.8)
Mean $I/\sigma(I)$	13.1 (2.4)
$R_{\rm merge}$ (%)	6.8 (32.4)

molecules per asymmetric unit, as predicted from the Matthews coefficient (Matthews, 1968).

3. Results

Recombinant *E. coli* CheA P3 dimerization domain (residues 279–332) with an N-terminal His_6 tag was overexpressed in *E. coli* as a soluble form, with a yield of over 50 mg purified protein per litre of culture. The best diffraction-quality crystals were obtained using a reservoir solution consisting of 28–36%(*v*/*v*) PEG 3350 and 0.1 *M* Tris pH 8.5. Crystals grew to approximate dimensions of 500 × 500 × 20 µm in one week (Fig. 2).

Crystals were transferred to a cryoprotectant solution from the hanging drop prior to exposing them to X-rays in a nitrogen cryostream. The diffraction images showed isotropic diffraction and resulted in complete collection of intensities in all resolution shells.

A set of 2.80 Å resolution diffraction data was collected using synchrotron radiation. A total of 28 102 unique reflections had an $R_{\rm merge}$ (on intensity) of 6.8%. The crystal belonged to the triclinic space group *P*1, with unit-cell parameters a = 59.271, b = 67.674, c = 82.815 Å, α = 77.568, β = 86.073, γ = 64.436°. The presence of up to ten dimeric four-helix bundles of P3 domains is plausible according to the Matthews coefficient. (When six to ten molecules are present in the asymmetric unit the Matthews coefficient ranges between 2.93 and 1.76 $Å^3\ Da^{-1}$, with a solvent content ranging between 58 and 30%, respectively. A solvent content of ∼30% is unlikely owing to the relatively low diffraction power of the crystal at the synchrotron.) The statistics for data collection are summarized in Table 1. The P3-domain structure from the *T. maritima* CheA (P3-P4-P5 domain) structure was used as a molecular-replacement model but failed to find a correct solution for the *E. coli* P3 domain. This may originate from the fact that closely packed helices can be difficult to distinguish by rotation or translation functions. Also, having more than six molecules in the asymmetric unit may reduce the chance of finding a correct solution. Hence, we plan to determine the structure by direct phasing methods and also to improve the diffraction resolution of the crystals.

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