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Crystallization of a complex between a novel C-terminal transmitter, HPt domain, of the anaerobic sensor kinase ArcB and the chemotaxis response regulator CheY

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Abstract

The histidine-containing phosphotransfer (HPt) domain at the C-terminus of the anaerobic sensor kinase ArcB has been cocrystallized with the chemotaxis response regulator CheY by a hanging-drop vapor-diffusion method. Crystals belong to space group $P2_12_12_1$ with unit-cell dimensions a = 55.32, b = 76.29 and c = 83.89 Å, with one molecule in the crystallographic asymmetric unit. The crystals diffract to 2.7 Å resolution. This is the first crystallization of a protein–protein complex formed by a transmitter domain of sensor kinase and a receiver domain of response regulator in the two-component signal-transduction system.

1. Introduction

The so-called 'two-component' system or His–Asp phosphorelay is one of intracellular signal-transduction systems involving phosphorylation reactions (Perkinson & Kofoid, 1992). Each system typically consists of two proteins, sensor kinase and response regulator. The phosphotransfer reactions in these proteins are mediated by two common characteristic modules, transmitter and receiver domains. Recently, it has been found that the histidine-containing phosphotransfer (HPt) domain is a new protein module with an active histidine residue that mediates phosphotransfer reactions in the His–Asp phosphorelays, as well as the typical transmitter (Kato, Mizuno, Shimizu & Hakoshima, 1997). It is present in a class of bacterial sensor kinases (Ishige, Nagasawa, Tokishita & Mizuno, 1994; Uhl & Miller, 1996) and is now being found in eukaryotic signaling proteins (Posas, Wurgler-Murphy, Maeda, Thai & Saito, 1996). A multi-step phosphorelay involving the HPt domain has been suggested for these signaling pathways (Appleby, Parkinson & Bourret, 1996). The anaerobic sensor kinase ArcB of *Escherichia coli* contains the HPt domain at the C-terminus. The crystal structure of this domain was determined at 2.06 Å resolution (Kato, Mizuno, Shimizu & Hakoshima, 1997). Recently, it has been shown by *in vitro* experiments (Tsuzuki, Ishige & Mizuno, 1995; Yaku, Kato, Hakoshima & Mizuno, 1997) that the HPt domain of ArcB phosphorylates the chemotaxis response regulator CheY, a non-recognizable response regulator for ArcB, with an efficiency comparable to that of the recognizable response regulator ArcA but scarcely phosphorylates another non-recognizable response regulator OmpR that regulates osmotic adaptation. All receiver domains identified to date are believed to fold into the same $(\alpha/\beta)_5$ fold (Volz, 1993). There is, as yet, little experimental evidence that defines the determinants of specificity needed to identify the appropriate receiver domain in any system. In particular, there is no experimental data available for the molecular recognition of a receiver domain by a transmitter domain at an atomic resolution. To clarify the molecular recognition of these response regulators by the HPt domain at an atomic level, we have undertaken the cocrystallization of the HPt domain with CheY. This is the first crystallization of a protein–protein complex formed by a transmitter domain of sensor kinase and a receiver domain of response regulator in the two-component signal-transduction system. Knowledge of the tertiary structure of the complex is important for understanding the physical basis of molecular recognition of the response regulator by the sensor kinase.

2. Materials and methods

Construction of *E. coli* strain and plasmid of the HPt domain of ArcB was described previously (Ishige, Nagasawa, Tokishita & Mizuno, 1994; Tsuzuki, Ishige & Mizuno, 1995; Nagase, Tokishita, Aiba & Mizuno, 1992). Purification of the HPt domain was carried out according to the method described (Kato, Ishige, Mizuno, Shimizu & Hakoshima, 1996). The HPt domain consists of 125 residues from Thr654 to Lys778 of ArcB, CheY, consisting of 128 amino-acid residues, was purified from *E. coli* cells carrying a multicopy of plasmid, pT7-CheY, in which the *cheY* gene is placed under the control of the phage-T7 promoter, by the method similar to those used (Kato, Ishige, Mizuno, Shimizu & Hakoshima, 1996). The cells were grown in Luria broth containing 50 μg ml^{-1} ampicillin and were cultured until late-logarithmic grown phase. Cells were suspended in 100 m*M* sodium phosphate buffer (pH 7.1) containing 5 m*M* $MgSO_4$, 2 m*M* 2-mercaptoethanol and 0.1 m*M* phenylmethanesulfonyl fluoride and were disrupted using a Bead Beader (Waken-yaku) at 277 K. The suspension was centrifuged, and solid ammonium sulfate was added in the supernatant up to 50% saturation. Again, the solution was centrifuged, and solid ammonium sulfate was added up to 75% saturation in the supernatant. Precipitates formed were recovered and dissolved in 20 m*M* Tris–HCl buffer (pH 7.8) containing 0.5 m*M* EDTA and 2 m*M* 2-mercaptoethanol (buffer *A*), and dialyzed twice against the same buffer. The sample was applied onto a DEAE column (Tosoh DEAE-Toyopearl 650M) equilibrated previously with buffer *A*. Protein was eluted with a linear NaCl gradient. Fractions containing CheY were eluted at 100 m*M* NaCl and were applied onto a gel-filtration column Sephacryl S-100. Then, the fractions containing CheY were further applied onto an affinity column with Affigel-Blue (BioRad) under the same buffer conditions as described above. The purified protein was concentrated to 63 mg ml^{-1} for crystallization. At every step, the protein was monitored by 17.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and gels were stained with Coomassie Brilliant Blue.

Crystallization of the complex was carried out by the hanging-drop vapor-diffusion technique (McPherson, 1976). Preliminary X-ray diffraction experiments were carried out with an imaging-plate area detector (Rigaku R-AXIS IIc) using Cu $K\alpha$ radiation (λ = 1.54178 Å) generated by a rotating-anode generator (Rigaku RU-300) operating at 40 kV and 100 mA. Intensity data for the structural analysis were collected at the BL-18B beamline station of the Photon Factory, Tsukuba. The data were recorded using 1 Å radiation, with 200 µm collimated beam, on a 200 × 400 mm Fuji imaging plate with a Weissenberg camera. The type 3A film cassette used holds one imaging plate that had a crystal-to-film distance of 429.78 mm. The maximum resolution that could be recorded in this configuration was 2.2 Å at the top and bottom edges and 4.4 Å at the right and left edges. Oscillations of ω of 10.0° were used with a speed of 2° s^{-1}. The coupling constant for sliding the cassette, ωZ, was 2 or 2.2° mm^{-1}. Two sets of data with the a and b axis settings were collected. A total of 26 Weissenberg photographs was taken each with exposure time of 100 or 150 s and overlap of ω of 0.5°. During data collection, the crystal was slid along the rotation spindle to shift the irradiation point on the crystal. The film images were read by a Fuji BAS2000 system for production of digitized data. Intensities were evaluated with the *DENZO* program (Otwinowski, 1993).

3. Results and discussion

The complex crystals of the HPt domain of ArcB and CheY were obtained at 277 K by vapor diffusion of 12.5 µl of hanging drops against 0.5 ml of reservoir of 30% polyethylene glycol (PEG) 4000 containing 200 m*M* ammonium sulfate. The protein solution contains 12.8 mg ml^{-1} of each of the HPt domain and CheY with 80 m*M* ammonium sulfate, 20 m*M* NaCl, 10 m*M* $MgCl_2$ and 12% PEG 4000. Thin rod crystals appeared within 1 d and grew to 20 × 50 × 500 µm (Fig. 1). The crystals were thoroughly washed with the reservoir and dissolved in an aliquot of 20 m*M* Tris–HCl (pH 7.8) buffer. Then, the solution was analyzed by 10% native polyacrylamide gel electrophoresis so as to determine the molar ratio of the HPt domain to CheY in the complex crystals. The gels stained with Coomassie Brilliant Blue showed that the crystals are the 1:1 complex of the HPt domain and CheY (Fig. 2).

Preliminary X-ray diffraction experiments showed that the crystals belong to the orthorhombic system, space group $P2_12_12_1$, with the unit-cell dimensions a = 55.32, b = 76.29 and c = 83.89 Å. Diffraction data were collected to 2.7 Å resolution with a completeness of 92.2% and an R_{merge} of 9.70%. For the highest resolution shell (3.0–2.7 Å), the completeness and R_{merge} are 84.2 and 36.1%, respectively. This crystal was estimated to contain one 1:1 complex in the asymmetric unit with a V_m value of 3.2 $Å^3$ Da^{-1} (Matthews, 1968). Structure analysis of the crystal used the molecular replacement methods. The rotation searches utilized both the direct rotation function, as implemented in *X-PLOR* (DeLano & Brünger, 1995), and the fast rotation function of *AMoRe* (Navaza, 1994) with the structures of ArcB HPt domain (Kato, Mizuno, Shimizu & Hakoshima, 1997) and CheY (Volz & Matsushima, 1991). All of the searches used reflections from 20 to 3 Å resolution. The orientations of both molecules that gave the top three peaks of the direct rotation function or the rotation function were followed by translation searches. Inspection of crystal packing of the solutions with the program *O* (Jones, Zou, Cowan & Kjeldgaard, 1991) is currently under way.

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Fig. 1. Crystal of the complex between ArcB HPt domain and CheY. The scale bar is 500 µm long.

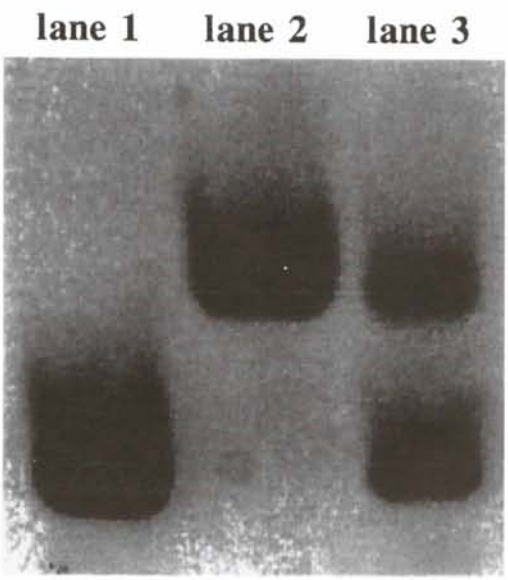

Fig. 2. 10% native polyacrylamide gel electrophoresis of ArcB HPt domain (lane 1), CheY (lane 2) and the crystal (lane 3).

References

Appleby, J. L., Parkinson, J. S. & Bourret, R. B. (1996). *Cell*, **86**; 845–848.
DeLano, W. L. & Brünger, A. T. (1995). *Acta Cryst.* D**51**, 740–748.
Ishige, K., Nagasawa, S., Tokishita, S. & Mizuno, T. (1994). *EMBO J.* **13**, 5195–5202.
Jones, T. A., Zou, J.-Y., Cowan, S. W. & Kjeldgaard, M. (1991). *Acta Cryst.* A**47**, 110–119.
Kato, M., Ishige, K., Mizuno, T., Shimizu, T. & Hakoshima, T. (1996). *Acta Cryst.* D**52**, 1214–1215.
Kato, M., Mizuno, T., Shimizu, T. & Hakoshima, T. (1997). *Cell*, **88**, 717–723.
McPherson, A. Jr (1976). *Methods Biochem Anal.* **23**, 249–345.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nagase, S., Tokishita, S., Aiba, H. & Mizuno, T. (1992). *Mol. Microbiol.* **6**, 799–807.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Otwinowski, Z. (1993). *Data Collection and Processing*, edited by L. Sawyer, N. W. Isaacs & S. Bailey, pp. 71–79. Warrington: Daresbury Laboratory.
Perkinson, J. S. & Kofoid, E. C. (1992). *Annu. Rev. Genet.* **26**, 71–112.
Posas, F., Wurgler-Murphy, S. M. Maeda, T., Thai, E. C. & Saito, H. (1996). *Cell*, **86**, 865–875.
Tsuzuki, M., Ishige, K. & Mizuno, T. (1995). *Mol. Microbiol.* **18**, 953–962.
Uhl, M. A. & Miller, J. F. (1996). *EMBO J.* **15**, 1028–1036.
Volz, K. (1993). *Biochemistry*, **32**, 11741–11753.
Volz, K. & Matsushima, P. (1991). *J. Biol. Chem.* **266**, 15511–15519.
Yaku, H., Kato, M., Hakoshima, T. & Mizuno, T. (1997). *FEBS Lett.* **408**, 337–340.