Acta Cryst. (1996). D52, 1214–1215

Crystallization and preliminary X-ray analysis of a histidine kinase domain of the anaerobic sensor protein ArcB from *Escherichia coli*

MASATO KATO,[a] KAZUYA ISHIGE,[b] TAKESHI MIZUNO,[b] TOSHIYUKI SHIMIZU[a] AND TOSHIO HAKOSHIMA[a]* at [a]*Department of Molecular Biology, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-01, Japan, and* [b]*School of Agricultural Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan. E-mail: hakosima@bs.aist-nara.ac.jp*

(Received 28 March 1996; accepted 3 July 1996)

Abstract

Crystals of a novel histidine protein kinase domain of the anaerobic sensor protein ArcB from *Escherichia coli* have been obtained by a hanging-drop vapor-diffusion method with micro- and macroseeding techniques. Preliminary X-ray crystallographic analysis revealed that they belong to space group $P2_12_12_1$ with dimensions $a = 30.56$, $b = 34.93$ and $c = 110.78$ Å, having one molecule in the crystallographic asymmetric unit. The crystals diffract to at least 2.0 Å resolution.

1. Introduction

Cells have many intracellular signal transduction systems involving phosphorylation reactions. In particular, a family of two-component systems is widely involved in the regulation of gene expression and cell behavior in bacterial cells. It has been estimated that 40–50 of these two-component regulatory systems exist in *Escherichia coli* (*E. coli*) cells. Each system typically consists of two proteins, the membrane sensor protein to the environment and the cognate regulator protein to control the target. The sensor and regulator proteins contain two common and characteristic modules, transmitter and receiver, respectively (Stock, Stock & Mottonenn, 1990; Parkinson & Kofoid, 1992; Alex & Simon, 1994). In a typical case, a sensor protein contains a C-terminal transmitter module preceded by a N-terminal signal-input domain and a regulator protein contains a N-terminal receiver module followed by a C-terminal signal-output domain. The transmitter module has an invariant autophosphorylated histidine residue and functions as a protein kinase of which the active residue is the histidine residue. Recent studies have shown that another variation of the two-component system exists in yeast (Ota & Varshavsky, 1993; Maeda, Wurgler-Murphy & Saito, 1994) and also in plants (Chang, Kwok, Bleecker & Meyerowitz, 1993).

Aerobic conditions allow *E. coli* cells to express operons encoding enzymes involved in aerobic metabolism (Iuchi & Lin, 1988, 1991; Iuchi, Cameron & Lin, 1989; Lin & Iuchi, 1991). The enzymes include the glyoxylate shunt, the pathways for fatty-acid degradation and several dehydrogenases of the flavoprotein class. The two-component system of ArcB/ArcA anaerobically represses expression of operons encoding these enzymes. Under microaerobic growth conditions, the system is also known to activate several operons, such as *cyd* encoding the cytochrome *d* complex and *pfl* encoding pyruvate formate lyase (Iuchi, Chepuri, Fu, Gennis & Lin, 1990; Fu, Iuchi & Lin, 1991; Sawers & Suppmann, 1992). ArcB belongs to a subclass of the sensor family that atypically contains two transmitter modules (Ishige, Nagasawa, Tokishita & Mizuno, 1994). Upon stimulation, ArcB first undergoes autophosphorylation at His292 of the N-terminal transmitter module. The phosphoryl group is then transferred to the receiver module of ArcA that functions as a transcription factor to the operons described above. The C-terminal transmitter module ($ArcB^C$), which consists of 125 residues with a molecular mass of 13.8 kDa, contains His717 as the active residue. This module shows no obvious sequence homology with any other kinases. Looking for weak similarities, it is possible to identify a very limited homology with C-terminal domains of a class of sensor proteins including BarA and EvgS of *E. coli* (Ishige *et al.*, 1994). It has been demonstrated that $ArcB^C$ can phosphorylate and activate OmpR as well as ArcA (Tsuzuki, Ishige & Mizuno, 1995). OmpR is the response regulator corresponding to the sensory kinase EnvZ, and the EnvZ/OmpR system regulates the expression of the *E. coli* outer membrane proteins (OmpF and OmpC) in response to an osmotic stimulus (Mizuno & Mizushima, 1990). This suggests that the C-terminal transmitter module may play an important role for signal transduction with specificity for receivers other than ArcA.

In this paper, we report purification, crystallization and preliminary X-ray crystallographic studies of $ArcB^C$.

2. Materials

Construction of the *E. coli* strain and plasmid used in this study were described previously (Nagasawa, Tokishita, Aiba & Mizuno, 1992; Ishige *et al.*, 1994). *E. coli* K-12 strain DZ225 (F^-, *ΔenvZ*, *ΔlacU*169, *araD*139, *rpsL*, *relA*, *thiA*, *flbB*, *ompC-lacZ*) harboring plasmid pSU2DH, which carries a gene encoding $ArcB^C$ under the *lac* promoter of *E. coli* on the pUC119 vector, was grown in 50 ml of Luria broth medium containing 200 μg ml^{-1} ampicillin until the OD_{660} was about 0.4–0.6 at 310 K. The culture was added to 2 l of Superbroth containing 200 μg ml^{-1} ampicillin and cultured until late-logarithmic grown phase. $ArcB^C$ protein was purified by methods similar to those previously reported (Ishige *et al.*, 1994). Cells (30 g) were suspended in 130 ml of sodium phosphate buffer (100 m*M*, pH 7.1) containing 5 m*M* $MgSO_4$, 2 m*M* 2-mercaptoethanol, and 0.1 m*M* phenylmethanesulfonyl fluoride and were stirred with glass beads ($\varphi = 0.1$ mm) for 1 min by a Bead Beater (Wakenyaku) five times at 277 K. The suspension was centrifuged for 30 min at 48 000*g* at 277 K. In the supernatant, solid ammonium sulfate was added up to 50% saturation and centrifuged for 30 min at 48 000*g*. Again, solid ammonium sulfate was added up to 70% saturation and centrifuged for 30 min at 48 000*g*. The precipitates formed were recovered and dissolved in 10 ml of a buffer solution containing 50 m*M* Tris–HCl (pH 7.8), 0.5 m*M* EDTA and 2 m*M* 2-mercaptoethanol, and dialyzed twice against 2 l of the same buffer. The sample was applied onto a DEAE column

ISSN 0907-4449

(2.5 × 10 cm, Tosoh DEAE–Toyopearl 650M) equilibrated previously with Tris–HCl buffer (pH 7.8). Protein was eluted with a 400 ml linear NaCl gradient from 0 to 350 m*M*. Fractions containing ArcB^c were eluted at ~ 100 m*M* NaCl and were applied onto a gel-filtration column Sephacryl S-100 (2.5 × 60 cm, Pharmacia-LKB). ArcB^c was eluted at ~ 170 ml of the same Tris–HCl buffer. Then, the fractions were further applied onto an high-performance liquid chromatography column system with MonoQ (1.0 × 10 cm, Pharmacia–LKB) under the same buffer conditions as described above. ArcB^c-containing fractions were eluted at ~ 100 m*M* NaCl. The fractions were concentrated to 63.8 mg ml^{-1} after changing the buffer to 5 m*M* Bis–Tris buffer (pH 7.0) containing 100 m*M* NaCl and stored at 277 K. At every step, the protein was monitored by 17.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and gels were stained with Coomassie Brilliant Blue.

3. Results and discussion

The crystals of ArcB^c were obtained in two weeks at 277 K by vapor diffusion of 10 µl of hanging drops against 1 ml of reservoir of 13% polyethylene glycol methyl ether 550. The protein solution contains 10.6 mg ml^{-1} ArcB^c with 50 m*M* acetic acid/sodium acetate buffer (pH 4.1), 5 m*M* $ZnSO_4$ and 12.5% polyethylene glycol methyl ether 550. To obtain these crystals reproducibly, the microseeding technique was used. Crystals appeared within 1 d by the technique and grew to 0.1 mm within 3 d. These crystals were used for the macro-seeding technique to obtain larger crystals. With this technique, the crystals with the maximum size of 0.2 × 0.3 × 0.5 mm were obtained for a week (Fig. 1). The crystals belong to the orthorhombic system, space group $P2_12_12_1$, with the unit-cell dimensions $a = 30.56$, $b = 34.93$, $c = 110.78$ Å.

X-ray diffraction data were collected at 277 K with an imaging-plate area detector (Rigaku R-AXIS IIc) using Cu $K\alpha$ radiation ($\lambda = 1.54178$ Å) generated by a rotating-anode generator (Rigaku RU-300) operating at 40 kV and 100 mA. The focus size of the X-ray beam was 0.3 mm and the distance from a crystal to an imaging plate was 100 mm. The data of 105° rotation were collected at a rate of 1.5° per 25 min for each image. Intensities were evaluated with the *PROCESS* program (Rigaku). Diffraction data were collected at 2.06 Å resolution with a completeness of 92.3% and an R_{merge} of 4.50%. This crystal was estimated to contain one molecule in the asymmetric unit with a V_m value of 2.1 Å^3 Da^{-1} and a V_{solv} value of 0.41 (Matthews, 1968). Structure analysis of the crystal using the multiple isomorphous replacement method with Hg-atom derivatives is in progress.

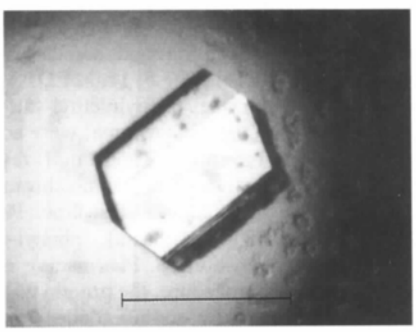

Fig. 1. Crystal of ArcB^c. The scale bar is 0.5 mm long.

This work was supported by Grants in Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture of Japan to TM (06276105) and TH (06276104, 08249225). MK was supported by a research fellowship from the Japan Society for the Promotion of Science.

References

Alex, L. A. & Simon, M. I. (1994). *Trends Genet.* **10**, 133–138.
Chang, C., Kwok, S. F., Bleecker, A. B. & Meyerowitz, E. M. (1993). *Science*, **262**, 539–544.
Fu, H. A., Iuchi, S. & Lin, E. C. C. (1991). *Mol. Gen. Genet.* **226**, 209–213.
Ishige, K., Nagasawa, S., Tokishita, S. & Mizuno, T. (1994). *EMBO J.* **13**, 5195–5202.
Iuchi, S., Cameron, D. C. & Lin, E. C. C. (1989). *J. Bacteriol.* **171**, 868–873.
Iuchi, S., Chepuri, V., Fu, H. A., Gennis, R. B. & Lin, E. C. C. (1990). *J. Bacteriol.* **172**, 6020–6025.
Iuchi, S. & Lin, E. C. C. (1988). *Proc. Natl Acad. Sci. USA*, **85**, 1888–1892.
Iuchi, S. & Lin, E. C. C. (1991). *Cell*, **66**, 5–7.
Lin, E. C. C. & Iuchi, S. (1991). *Annu. Rev. Genet.* **25**, 361–387.
Maeda, T., Wurgler-Murphy, S. M. & Saito, H. (1994). *Nature (London)*, **369**, 242–245.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mizuno, T. & Mizushima, S. (1990). *Mol. Microbiol.* **4**, 1077–1082.
Nagasawa, S., Tokishita, S., Aiba, H. & Mizuno, T. (1992). *Mol. Microbiol.* **6**, 799–807.
Ota, I. M. & Varshavsky, A. (1993). *Science*, **262**, 566–569.
Parkinson, J. S. & Kofoid, E. C. (1992). *Annu. Rev. Genet.* **26**, 71–112.
Sawyers, G. & Suppmann, B. (1992). *J. Bacteriol.* **174**, 3474–3478.
Stock, J. B., Stock, A. M. & Mottonenn, J. M. (1990). *Nature (London)*, **344**, 395–400.
Tsuzuki, M., Ishige, K. & Mizuno, T. (1995). *Mol. Microbiol.* **18**, 953–962.