Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The MinD protein from the hyperthermophilic archaeon *Pyrococcus horikoshii*: crystallization and preliminary X-ray analysis

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Received 4 January 2001
Accepted 2 April 2001

MinD is one of the proteins regulating cell division. MinD from *Escherichia coli* has been designated as a type of motor protein which has an ATPase activity. This paper deals with the first crystallization and preliminary crystallographic analysis of recombinant MinD from *Pyrococcus horikoshii* (molecular weight 26.3 kDa) expressed in *E. coli*. Crystals of MinD were obtained by the hanging-drop vapour-diffusion method. MinD crystals belong to space group $P2_13$, with unit-cell parameters $a = b = c = 98.5$ Å, and diffract to 3.0 Å resolution. The asymmetric units each contain one molecule of MinD, giving a crystal volume per protein mass (V_M) of 3.0 Å^3 Da^{-1} and a solvent content of 59.0%.

1. Introduction

Proper placement of cell-division sites is critical for the replication of all living cells. With the recent advances in fluorescence microscopy and immunoelectron microscopy, the mechanisms of cell division have become a hot topic in cell biology (Rothfield *et al.*, 1999; Jacobs & Shapiro, 1999; Sullivan & Maddock, 2000). To accurately place the division site in the middle of the *E. coli* cell, the site-specific inhibition of potential division sites is required. The cooperative actions of *min* proteins MinC, MinD and MinE mediate this inhibition (de Boer *et al.*, 1989). Therefore, the positioning of *min* proteins is critical to regulating the placement of the cell-division apparatus.

Selecting division sites in *E. coli* cells involves the interdependent regulation of the MinC–MinD complex (MinCD) and MinE. In *E. coli* cells, MinCD oscillates from pole to pole rapidly in a MinE-dependent manner (Raskin & de Boer, 1999*a*,*b*; Hu & Lutkenhaus, 1999; Rowland *et al.*, 2000). Conversely, the formation of the MinE ring at the cell centre requires MinD but is independent of MinC (Raskin & de Boer, 1997). MinE's ability to disrupt the MinCD complex depresses the activity of MinCD's cell-division inhibitor near the cell centre (Huang *et al.*, 1996). Recently, the solution structure of the *E. coli* MinE homodimer was solved (King *et al.*, 2000). The structure shows that anti-CD domains of MinE are present on either side of the MinE dimer. Subsequently, the FtsZ ring that forms adjacent to the MinE ring allows cell division to commence (Addinall & Lutkenhaus, 1996). However, the mechanism by which MinCD prevents the formation of the FtsZ ring is not yet known.

In recent immunoelectron microscopy and biochemical studies, it was found that MinD is a membrane ATPase needed for the correct placement of the division site in bacterial cells (de Boer *et al.*, 1991). This research also indicated that MinD is the primary oscillatory motor that recruits MinC (Raskin & de Boer, 1999*b*). The binding and hydrolysis of ATP by MinD may provide the motive force for the MinCD complex.

As the first step toward understanding the cell-division mechanism at an atomic resolution, we report here the gene cloning, overexpression, crystallization and preliminary X-ray crystallographic analysis of *P. horikoshii* MinD, which comprises 245 amino-acid residues (MW = 26.3 kDa).

2. Cloning, overexpression and purification

The gene encoding *P. horikoshii* MinD (PH0612; Kawarabayasi *et al.*, 1998) was amplified by PCR and cloned into a pGEM-T Easy vector (Promega). The MinD-coding insert DNA was digested with *Nde*I and *Eco*RI and ligated into a pET-22b(+) vector (Novagen). The *E. coli* cells BL21-CodonPlus(DE3)-RIL (Stratagene) were transformed with the pET-22b(+)/MinD plasmid. The cells were grown at 310 K in 6 l LB medium containing 50 µg ml^{-1} ampicillin and 34 µg ml^{-1} chloramphenicol.

The expression of MinD was induced by 1 m*M* IPTG. After IPTG injection, the medium was incubated at 310 K for 3 h with shaking. The cells were harvested by centrifugation at 4000*g* for 15 min at 277 K and resuspended in STE buffer (50 m*M* Tris–HCl

pH 8.0, 1 m*M* EDTA, 50 m*M* NaCl) containing 1 m*M* dithiothreitol and 1 m*M* phenylmethylsulfonyl fluoride. The cells were disrupted by a French press at 8.3 MPa. The homogenate was clarified by centrifugation at 40 000*g* for 30 min at 277 K. The supernatant of the cell extracts was incubated at 343 K for 30 min and centrifuged at 40 000*g* for 30 min. After the centrifugation, 1%(*v*/*v*) Polymin P was added to the supernatant, which was then stirred for 30 min at 277 K and centrifuged at 40 000*g* for 30 min at 277 K. The supernatant was mixed slowly to 60% saturation in ammonium sulfate and was centrifuged at 20 000*g* for 30 min. The protein pellet was resuspended in buffer *A* (20 m*M* Tris–HCl pH 8.0, 1 m*M* EDTA, 50 m*M* NaCl) and dialyzed against buffer *A*. The protein solution was filtrated with a 0.22 µm filter and applied to a HiPrep 16/10 Q-XL column (Amersham Pharmacia Biotech) equilibrated with buffer *A*. After washing with buffer *A*, the bound protein was eluted using a linear gradient of 0.05–1.0 *M* NaCl in 400 ml buffer. The fractions containing MinD were pooled and concentrated to 10 ml and loaded onto a HiLoad 26/60 Superdex 200pg column (Amersham Pharmacia Biotech) equilibrated with buffer *B* (20 m*M* Tris–HCl pH 8.0, 1 m*M* EDTA, 200 m*M* NaCl). The protein eluted as a single peak. The fractions containing MinD were pooled and dialyzed against Milli-Q and concentrated by ultrafiltration using CentriPlus-10 and Centricon-10 microconcentrators (Amicon Inc.) to a final concentration of 10 mg ml^{-1}. The purity of the protein was analyzed by MALDI-TOF mass spectrometry (Voyager DE-PRO, PerSeptive Biosystems).

3. Crystallization and data collection

All crystallization experiments were performed using the hanging-drop vapour-diffusion method in a 24-well tissue-culture Linbro plate at 293 K. The initial crystallization trials were carried out using reservoir solutions consisting of 0.5 ml Hampton Research Crystal Screen (Jancarik & Kim, 1991) or Grid Screen. Each drop contained 1 µl reservoir solution and 1 µl protein solution. Crystals were obtained within 36 h from condition 1 of Crystal Screen I (0.1 *M* sodium acetate pH 4.6, 30% MPD, 0.02 *M* $CaCl_2$) and condition D6 of Grid Screen MPD (0.1 *M* Bicine pH 9.0, 65% MPD). Further trials optimized these conditions and improved crystals (Fig. 1) were obtained with 0.1 *M* sodium acetate pH 4.3–4.5, 30% MPD, 0.02 *M* $CaCl_2$.

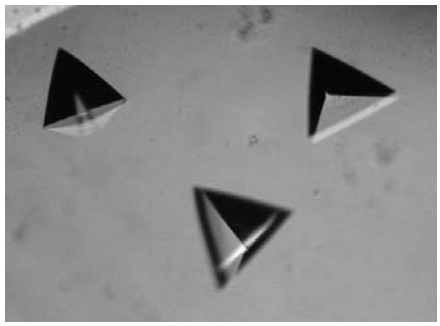

Figure 1
Crystals of MinD grown by the hanging-drop method. The average dimensions of these crystals were 300 × 300 × 300 µm.

Preliminary X-ray diffraction data were collected from cryocooled (100 K) crystals on a DIP-R300 image-plate system using an M18XHF X-ray generator with Cu $K\alpha$ radiation (MAC Science) operating at 50 kV and 90 mA. Data were collected at 1.0° oscillation with the crystal-to-detector distance set to 150 mm. Data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The merged data set is 94.0% complete to 3.0 Å, with an $R_{\rm merge}$ (on intensity) of 9.1%. The space group has been assigned as $P2_13$. The unit-cell parameters are $a = b = c = 98.5$ Å. The asymmetric unit contains one molecule of MinD, giving a crystal volume per protein mass ($V_{\rm M}$) of 3.0 Å^3 Da^{-1} and a solvent content of 59.0%.

We will subsequently perform trials for the overexpression, purification and crystallization of Se-Met MinD. The structure will be solved using the multiwavelength anomalous dispersion (MAD) method with Se-Met MinD.

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