Direct Monitoring of Interaction between *Escherichia coli* Proteins, MinC and Monomeric FtsZ, in Solution

Takashi Okuno,†a Machiko Ogoh, b Hiromitsu Tanina, b Noriaki Funasaki, b and Kentaro Kogure b

a Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama; 2630 Sugitani, Toyama 930–0194, Japan; and b Department of Biophysical Chemistry, Kyoto Pharmaceutical University; 5 Nakachi-cho,Misasagi, Yamashina-ku, Kyoto 607–8414, Japan. Received March 3, 2009; accepted May 14, 2009; published online May 19, 2009

MinC plays an important role in regulation of the cell division site in *Escherichia coli*. Previous studies using sedimentation and electron microscopic methods suggested that MinC interacts with the FtsZ polymer and inhibits further FtsZ polymerization. However, it is difficult to clarify details regarding specific molecular interactions by such static analytic methods. In this study, a fluorescence resonance energy transfer (FRET) method was developed to directly observe the interaction between Cy3-labeled MinC and Cy5-labeled FtsZ in solution. FRET analysis indicated that MinC interacts with monomeric rather than polymeric FtsZ in solution. This suggests that interactions between monomeric FtsZ and MinC are important for controlling of FtsZ polymerization by MinC.

**Key words** fluorescence resonance energy transfer; cell division; inhibitor; FtsZ; MinC

FtsZ, which is an essential participant in prokaryotic cell division, forms a filamentous ring structure that involves guanosine 5'-triphosphate (GTP)-dependent polymerization at the mid cell before division in *Escherichia coli* (*E. coli*). 1–6 It is known that polymerization and depolymerization of FtsZ polymer occur frequently in vivo. 7 It has been indicated that small molecular compounds, which inhibit FtsZ activity, have a potential as novel antibacterial drugs. 8 Information about regulation mechanism of FtsZ polymerization should be useful for development of a new class of antibacterial drug. The stability of FtsZ polymer is regulated by several inhibitory and stimulatory proteins in vivo. 9,10 Moreover, inhibition of FtsZ polymerization by MinC protein is responsible for controlling the site of cell division. 11–13 Thus, MinC is recruited to the inner membrane surface near the cell pole under the control of MinD and MinE to prevent septum ring formation near the cell pole. 14 Recent studies using sedimentation and electron microscopic methods suggested that MinC interacts with the FtsZ polymer and inhibits further FtsZ polymerization. 15 However, details about the interaction between MinC and FtsZ were still unclear because no techniques had yet been developed for direct observation of molecular interaction between MinC and FtsZ in the reaction solution. The present study describes the development of a direct monitoring technique and its successful application to characterize the interaction between MinC and FtsZ in solution by fluorescence resonance energy transfer (FRET).

**MATERIALS AND METHODS**

**Bacterial Strains and Plasmids** DH5α and BL21(DE3) (NIPPN GENE, Japan) were purchased and used as host strains for construction of plasmids and for expression of genes cloned using plasmids. To construct the minC and ftsZ genes with attached histidine tags, the minC and ftsZ gene was amplified by polymerase chain reaction (PCR) using purified chromosomal DNA from *E. coli* as a template. The PCR product was inserted into the Nde–BamHI site in the multi-cloning site of pET-15b (Novagen, U.S.A.). Oligonucleotides used to amplify the minC and ftsZ genes are listed below. (minC: 5′-AGCGCTATGTCAAAACAGCCAATCGACGTT-3′ and 5′-GCCGATCTCTAATTTACGTT-GAAGCTGG-3′, ftsZ: 5′-GACGCTATGTGGTGAGAAAC-3′ and 5′-GCATGGAATCTTACC-3′).

**Purification of E. coli MinC and FtsZ** MinC was expressed in *E. coli* BL21 (DE3). Cells were grown at 37°C, and expression of MinC was induced by the addition of 1 mM isopropyl-β-D-thio galactopyranoside (IPTG) (Wako Pure Chemical, Japan) and incubated at 30°C for 3 h. Cells were harvested by centrifugation at 4°C, lysed by sonication on ice, and centrifuged for 20 min at 10000 rpm at 4°C. The supernatant was loaded on a Macro-Prep High Q Support column (Bio-Rad Laboratories, Inc, U.S.A.) in buffer A (25 mM Tris–HCl pH 8.0, 25 mM NaCl, 10% glycerol) and eluted with a linear gradient from 0 to 1 M NaCl. Fractions containing MinC proteins were loaded on a Ni Sepharose column (GE Healthcare, U.K.) equilibrated with buffer A. The MinC protein was eluted with buffer A plus 500 mM imidazole. Fractions containing MinC protein were collected and pooled and dialyzed against buffer A containing 1 mM dithiothreitol (DTT) at 4°C. Purified MinD protein was stored at −80°C. The MinC protein activity is retained for at least 1 month at −80°C. FtsZ was purified following the same protocol.

**Preparation of Fluorescent-Labeled Protein** Purified MinC was incubated with Cy3 Maleimide mono-reactive dye (GE Healthcare, U.K.) at 4°C in the dark for 3 h in reaction buffer (50 mM potassium phosphate buffer pH 7.0). Unreacted excess Cy3 dye was removed on a G-25 superdex column (GE Healthcare, U.K.) equilibrated with 25 mM Tris–HCl pH 8.0, 25 mM NaCl, and 10% glycerol. An FtsZ mutant (FtsZ-Cys), with a C-terminal attached cysteine residue, was labeled by Cy5 dye using the same protocol as that used for the MinC-Cy3 preparation. Purified Cy-dye labeled protein was stored at −80°C.

**Sedimentation Assay of FtsZ Polymerization** FtsZ (12 μg) was incubated in 50 μl of polymerization buffer

* To whom correspondence should be addressed. e-mail: okunot@pha.u-toyama.ac.jp

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(50 mM 2-morpholinoethane sulfonic acid (MES)-NaOH, pH 6.5, 50 mM KCl, 10 mM MgCl₂) with GTP at 37 °C. After incubation for 10 min, ultracentrifugation was carried out at 250000×g in a Beckman TLA100.1 rotor for 15 min. Supernatants were carefully removed and pellets were resuspended in polymerization buffer (50 μl). The supernatant and pellet samples were electrophoresed on sodium dodecyl sulfate (SDS)–12% polyacrylamide gel electrophoresis (PAGE) and stained with Coomassie brilliant blue.

**Spectroscopic Analysis** Emission spectra were measured at 27 °C on a fluorescence spectrophotometer F-2500 (Hitachi High-technologies Co., Japan). The excitation wavelength was 520 nm and emission spectra were recorded between 630—730 nm.

**RESULTS AND DISCUSSION**

To observe molecular interactions between MinC and FtsZ, we purified *E. coli* MinC and FtsZ with His-tag at their N-termini and confirmed that the purified MinC and FtsZ retained their activities. A conventional sedimentation assay was used to confirm their activities. FtsZ (12 μM) was mixed with different concentrations of MinC, and polymerization was initiated by addition of 1 mM GTP. In the presence of GTP, FtsZ polymerized and then accumulated in the pellet fraction by ultracentrifugation (Fig. 1A). The amount of FtsZ recovered in the pellet was reduced to the control amount of FtsZ in the pellet fractions in a concentration-dependent manner.

To investigate interactions between MinC and the FtsZ polymer, Min-Cy3 (6 μM) or FtsZ-Cy5 was incubated in the presence of 1 mM GTP in reaction buffer (50 mM MES-NaOH pH 6.5, 50 mM KCl, 1 mM MgCl₂) with increasing concentrations of MinC (0—12 μM) or MinC-Cy3. After 10 min incubation at 37 °C, the reaction solutions were centrifuged at 250000×g in a Beckman TLA100.1 rotor for 15 min. The supernatants (S) and pellets (P) samples were electrophoresed on SDS–12% PAGE and stained with Coomassie brilliant blue. Reproducibility of this experiment was confirmed at least three times.

**Fig. 1.** MinC Prevents FtsZ Polymerization in a Dose-Dependent Manner

The images are the SDS-PAGE analysis using A) unmodified proteins MinC and FtsZ, and B) fluorescent-labeled proteins MinC-Cy3 and FtsZ-Cy5. FtsZ (12 μM) or FtsZ-Cy5 was incubated in the presence of 1 mM GTP in reaction buffer (50 mM MES-NaOH pH 6.5, 50 mM KCl, 1 mM MgCl₂) with increasing concentrations of MinC (0—12 μM) or MinC-Cy3. After 10 min incubation at 37 °C, the reaction solutions were centrifuged at 250000×g in a Beckman TLA100.1 rotor for 15 min. The supernatants (S) and pellets (P) samples were electrophoresed on SDS–12% PAGE and stained with Coomassie brilliant blue. Reproducibility of this experiment was confirmed at least three times.

**Fig. 2.** Emission Spectra of MinC-Cy3 and FtsZ-Cy5 in the Presence or Absence of GTP

Emission spectra of MinC-Cy3, FtsZ-Cy5 and mixture were measured using λ=520 nm for excitation of Cy3 fluorescence. Gray broken line shows fluorescence of MinC-Cy3 (6 μM), gray line shows fluorescence of FtsZ-Cy5 (12 μM) and black line shows mixture of MinC-Cy3 (6 μM) and FtsZ-Cy5 (12 μM). Black broken line shows the calculated spectra of donor plus acceptor. The proteins were incubated in buffer (50 mM MES-NaOH pH 6.5, 50 mM KCl, 1 mM MgCl₂) at 27 °C. Reproducibility of this experiment was confirmed at least two times. (A) Fluorescent-labeled proteins were incubated in the presence of 1 mM GTP for 10 min. Before addition of MinC-Cy3, FtsZ-Cy5 was incubated with 1 mM for 10 min. (B) Fluorescent-labeled proteins were incubated without GTP for 10 min.

**Fig. 3.** Fluoroscence-labeled proteins MinC-Cy3 and FtsZ-Cy5 mixture were incubated with different concentrations of MinC or MinC-Cy3. After incubation, the calculated spectra of donor plus acceptor were obtained. The observed emission intensity of the acceptor (Cy5) was slightly higher than that of the theoretical emission spectrum calculated by adding the MinC-Cy3 and FtsZ-Cy5 spectra. The observed acceptor emission peak intensity should be the same as the calculated spectrum if MinC-Cy3 does not interact with FtsZ-Cy5. In the presence of the FtsZ polymer, FRET between MinC-Cy3 and FtsZ-Cy5 was observed. This suggested that MinC interacts weakly with the FtsZ polymer. To observe the interaction between MinC-Cy3 and the FtsZ-Cy5 monomer, the emission spectrum of a MinC-Cy3 and FtsZ-Cy5 mixture was recorded (Fig. 2B). Interestingly, the emis-
sion intensity of the acceptor was significantly enhanced relative to that for MinC plus the FtsZ polymer. The FRET signal observed for the mixture of MinC plus the FtsZ polymer was about 30% of that recorded for MinC plus the FtsZ monomer. These results indicate that MinC preferably interacts with the FtsZ monomer in solution. Up to the present, there were some reports regarding inhibition of FtsZ polymerization by MinC, but those information are not enough to understand molecular interaction between MinC and FtsZ. In this study, we succeeded in evaluation of interaction between MinC and FtsZ by FRET analysis in the solution. To understand the molecular mechanism by which MinC inhibits FtsZ polymerization, it is important to investigate the interaction between MinC and the monomeric FtsZ. Further studies of MinC and the FtsZ monomer are needed to understand the detailed mechanism by which MinC inhibits FtsZ polymerization.

Acknowledgement This work was partially supported by the 21st Century COE Program and by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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