Glutamate-induced Assembly of Bacterial Cell Division Protein FtsZ

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The polymerization of FtsZ is a finely regulated process that plays an essential role in the bacterial cell division process. However, only a few modulators of FtsZ polymerization are known. We identified monosodium glutamate as a potent inducer of FtsZ polymerization. In the presence of GTP, glutamate enhanced the rate and extent of polymerization of FtsZ in a concentration-dependent manner; ~90% of the protein was sedimented as polymer in the presence of 1 mM glutamate. Electron micrographs of glutamate-induced polymers showed large filamentous structures with extensive bundling. Furthermore, glutamate strongly stabilized the polymers against dilution-induced disassembly, and it decreased the GTPase activity of FtsZ. Calcium induced FtsZ polymerization and bundling of FtsZ polymers; interestingly, although 1 mM glutamate produced a larger light-scattering signal than produced by 10 mM calcium, the amount of polymer sedimented in the presence of 1 mM glutamate and 10 mM calcium was similar. Thus, the increased light scattering in the presence of glutamate must be due to its ability to induce more extensive bundling of FtsZ polymers than calcium. The data suggest that calcium and glutamate might induce FtsZ polymerization by different mechanisms.

FtsZ, a 40.3-kDa protein, is a key protein involved in prokaryotic cell division (1, 2). It forms the structural element called Z-ring at the site of cell division, and it remains associated with the inner face of the cytoplasmic membrane throughout the septation process (3). Homologs of FtsZ have been found in all free-living prokaryotes examined to date (4–6), including many species of Archaea. It has also been found in chloroplasts of higher plants, where it is involved in chloroplast division (7). Its wide distribution and high degree of sequence conservation suggest that it probably plays a similar role in all bacterial and archaeal species (8–10).

Consistent with its cytoskeletal role, FtsZ has several properties in common with the eukaryotic cytoskeleton protein, tubulin. Like tubulin, FtsZ binds and hydrolyzes GTP, and it polymerizes to form long tubules in a GTP-dependent manner (11–14). It has been shown that purified FtsZ polymerizes into structures that closely resemble those formed by purified tubulin (15). Furthermore, FtsZ shows limited but significant sequence similarity to tubulin. Sequence alignment of FtsZ and tubulin reveals that several structural glycine and proline residues and most of the residues that are involved in GTP binding are conserved. In addition, FtsZ contains the tubulin signature GTP binding sequence motif GG(11, 12, 16). Recently, the crystal structure of FtsZ from Methanococcus jannaschii has been determined (17). FtsZ has two domains arranged around a central helix. GTPase activity is localized in the amino terminus domain of the protein, whereas the carboxyl-terminal domain function is still unknown (17). The three-dimensional structure of FtsZ also shows striking similarities with α and β tubulin (18).

Furthermore, molecular phylogenetic data indicate that an archaeal FtsZ is the most tubulin-like of all prokaryotic FtsZ proteins found so far, suggesting that tubulin may have evolved from an ancestral FtsZ (5).

At low concentrations, purified FtsZ monomers polymerize into single-stranded protofilaments with little or no bundling of protofilaments in an assembly reaction that is believed to be isodesmic (non-cooperative) in nature (19). However, in the presence of DEAE-dextran (15, 20) or high concentrations of calcium (21–23) FtsZ monomers polymerize into long, rod-shaped or tubular polymers that become extensively bundled. The assembly reaction is stimulated, and resulting polymers are stabilized by these agents. These studies demonstrate that the extent and nature of the polymer formed highly depends on the reaction conditions. Interestingly, calcium exerts contrasting effects on the polymerization of FtsZ and microtubules. Calcium strongly inhibits tubulin assembly and depolymerizes microtubules, whereas it promotes FtsZ assembly and induces bundling of FtsZ filaments (23). Biochemical studies have been carried out to study the effect of various factors such as temperature and pH on FtsZ polymerization (24–26). Many inducers of tubulin polymerization have been identified over the years, and these modulators of polymerization have been extensively utilized to understand the molecular mechanism of microtubule polymerization dynamics (27); however, only a few inducers of FtsZ polymerization have thus far been identified.

In this study we found that monosodium glutamate is a potent inducer of FtsZ polymerization, and it stabilizes FtsZ polymers against disassembly. Electron micrographs showed that glutamate-induced FtsZ polymers form extensive bundles. Furthermore, glutamate decreased the GTPase activity of FtsZ. The property of filament bundling may be important in FtsZ function in the septation process. In addition, calcium and glutamate exerted differential effects on FtsZ polymerization, indicating that they modulate FtsZ polymerization through different mechanisms.

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**EXPERIMENTAL PROCEDURES**

**Materials**—Pipes, sodium glutamate, isopropyl-β-D-thiogalactopyranoside, and SE-52 were obtained from Sigma. DE-52 was obtained from Whatman International Ltd. All other chemicals used were of analytical grade.

**Purification of FtsZ—** Recombinant *Escherichia coli* FtsZ was overexpressed and purified from BL21 strain (a gift from Dr. H. P. Erickson, Duke University) as described earlier (28) with some modifications. Briefly, the cells were grown at 37°C in LB broth containing 50 μg/ml ampicillin until they reached early log phase (A600 ~ 0.4–0.5) when protein expression was induced by the addition of 0.4 mM isopropyl-β-D-thiogalactopyranoside for an additional 5 h. Cells were harvested by centrifugation at 12,000 × g for 30 min and suspended in lysis buffer (50 mM Tris (pH 8), 100 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.1% mercaptoethanol, and 1 mM MgSO4) on ice. Cells were lysed by sonication with 20-s pulses at 30-s intervals between the pulses for four cycles. Partially lysed cells were further disrupted using a Polytron homogenizer (Polytron PT-3000). Lysozyme (0.4 mg/ml) was added to the partially lysed cell suspension, which then was incubated on ice for 1 h. It was again incubated with 10 mM MgSO4 for 5 min, and the suspension was sonicated for 6 cycles with 20-s pulses. The insoluble debris was removed by centrifugation at 12,000 × g at 4°C for 30 min. The cell-free supernatant was saturated to 25% with ammonium sulfate solution and incubated for 90 min at 4°C. The protein suspension was centrifuged at 27,000 × g for 30 min. The pellet was resuspended in 50 mM Tris buffer (pH 8) and dialyzed against the same buffer for 4 h. Purification of FtsZ was further carried out using a DE-52 ion exchange column chromatography as described previously (13). Briefly, FtsZ was loaded on a DE-52 column pre-equilibrated with 50 mM Tris buffer (pH 8), and the protein was eluted with increasing concentrations of KCl. FtsZ-containing fractions were eluted at 250 mM KCl; protein concentrations were determined by the Bradford assay using bovine serum albumin as a standard. These fractions were pooled together and concentrated in a Centricon-10 concentrator. One cycle of temperature-dependent polymerization and depolymerization was then used to further purify the FtsZ. The polymerization reaction was carried out in buffer containing 25 mM Pipes (pH 6.8), 10 mM MgSO4, 10 mM CaCl2, 1 mM monosodium glutamate, and 1 mM GTP at 37°C for 30 min. FtsZ polymers were sedimented by centrifugation at 27,000 × g for 30 min at 37°C. The pellet was resuspended on ice in 25 mM Pipes buffer (pH 6.8), and the suspension was centrifuged at 12,000 × g for 15 min at 4°C to remove the insoluble aggregates. The FtsZ concentration was determined by the Bradford method using bovine serum albumin as a standard (29). FtsZ was frozen and stored at −80°C.

**Sedimentation Assay—** FtsZ (6 μM) was polymerized in buffer A containing 1 mM CaCl2 and the presence and absence of different concentrations of monosodium glutamate on ice. After the addition of 1 mM GTP, the sample was immediately placed in a cuvette at 37°C, and the polymerization reaction was monitored at 37°C by light scattering at 500 nm using a JASCO 6500 spectrophotometer. The extinction coefficient was 500 nm.

**Electron Microscopy—** FtsZ (6 μM) was polymerized in buffer A containing 1 mM CaCl2 and the presence and absence of different concentrations of sodium glutamate for 30 min at 37°C. The polymers were collected by sedimenting them at 27,000 × g for 30 min at 27°C. The protein concentration in the supernatant was measured by the Bradford assay using bovine serum albumin as a standard.

**Stability of FtsZ Polymers in the Presence of Glutamate—** FtsZ (1 mg/ml) was polymerized in buffer A with 10 mM calcium and 1 mM GTP at 37°C. The protein solution was then diluted 10-fold to reach a final FtsZ concentration of 0.05 mg/ml in different concentrations of warm glutamate. The reaction mixtures were incubated at 37°C for another 30 min and centrifuged at 50,000 × g for 30 min. The pellet obtained were dissolved in 30 μl of 25 mM Pipes buffer containing 0.5% SDS, and 20 μl of each sample was analyzed by 10% SDS-PAGE.

The intensity of the bands was determined by a gel documentation system obtained from Kodak Digital Science.

**GTP Hydrolysis—** A malachite green sodium molybdate assay was used to measure the production of inorganic phosphate during GTP hydrolysis (19, 30). Samples containing 6 μM FtsZ with different concentrations of glutamate were prepared in buffer A at 0°C, and 1 mM GTP was added to the reaction mixtures. Immediately, the reaction mixtures were transferred to 37°C and incubated for different lengths of time. The hydrolysis reaction was quenched at desired time points by the addition of 10% v/v 7 M perchloric acid to the reaction mixtures, and the quenched reaction mixtures were centrifuged for 5 min to remove aggregated proteins. Twenty microliters of the supernatants were incubated with 900 μl of filtered malachite green solution (0.045% malachite green, 4.2% ammonium molybdate, and 0.02% Triton X-100) at room temperature for 30 min, and the phosphate ions produced were determined by measuring the absorbance of samples at 650 nm. The reaction was normalized including a control without FtsZ. A phosphate standard curve was prepared using sodium phosphate.

**RESULTS**

**Purification of FtsZ by Glutamate-enhanced Polymerization—** FtsZ and its eukaryotic homolog tubulin are both known to undergo multiple temperature-dependent polymerization and depolymerization cycles in the presence of GTP (31). The polymerization of tubulin is well understood, and several inducers of tubulin assembly in vitro are known. The purification procedure for tubulin involves temperature- and GTP-dependent polymerization and depolymerization cycles followed by phosphocellulose chromatography, which not only increases the purity level of the protein but also increases the yield of the assembly-compotent fraction of tubulin (32, 33). Taking advantage of the tubulin literature, we sought to find inducers of FtsZ polymerization. We tried several well known agents that promote tubulin polymerization including dimethyl sulfoxide (DMSO), glutamate, taxol, and glutamate (34, 35). Glutamate increased the magnitude of the light-scattering signal, and an effect was seen on their effects on tubulin polymerization, 4 μM glycerol, 10% v/v Me2SO, and 10 μM taxol did not show any effect on FtsZ polymerization (data not shown). However, glutamate did enhance FtsZ assembly in a concentration range similar to the concentration range that enhances tubulin polymerization (Fig. 1). Several groups have routinely purified FtsZ from crude extracts using a cation exchange (DE-52) chromatographic procedure (13, 28). Thus, similar to the purification procedure used for glutamate, we introduced glutamate into a temperature-dependent polymerization and depolymerization cycle after the DE-52 column to further purify FtsZ while keeping its activity (Experimental Procedures). The combination of a cycle of polymerization and depolymerization with DE-52 chromatography yielded FtsZ of high purity (>98%) as analyzed by Coomassie-stained SDS-PAGE (data not shown).

**Glutamate-induced Polymerization of FtsZ—** Our goal was to discover modulators of FtsZ polymerization, which could be further used to understand the basic mechanisms of FtsZ polymerization and the roles of FtsZ polymerization in bacterial cell division. Monosodium glutamate increased the rate and extent of FtsZ polymerization in a concentration-dependent manner with an optimal effect at a concentration of 1 mM (Fig. 1A). To further demonstrate that glutamate is a potent inducer of FtsZ polymerization, the rate and extent of FtsZ (8 μM) polymerization was first monitored for 10 min by light scattering in the absence of glutamate. After 10 min, 1 mM glutamate was added to the cuvette from a stock of 2 mM warm glutamate, and the polymerization reaction was monitored for an additional 15 min. The addition of 1 mM warm glutamate strikingly increased the magnitude of the light-scattering signal, and an apparent steady state was achieved quickly (Fig. 1B).

To verify whether the increase in the light-scattering signal was due to a genuine increase in the FtsZ polymerization, we measured the mass of polymeric FtsZ in the presence of differ-
ent concentrations of glutamate (Fig. 2). The FtsZ polymer mass clearly increased with increasing concentrations of glutamate. Between 85 and 92% of the initial protein could be sedimented as polymer mass in the presence of 1 M glutamate. To find out whether glutamate-induced FtsZ polymerization was temperature-dependent or not, 6 μM FtsZ was incubated with 1 M glutamate plus 1 mM GTP in buffer A for 30 min at 4 °C. After incubation, the reaction mixture was centrifuged at 4 °C as previously described in Fig. 2. Then the protein concentration of the supernatant was measured, and it was found to be identical with the total protein concentration used in the assay (data not shown). The result showed that no glutamate-induced polymerization occurred at 4 °C. However, ~90% of the total protein formed sedimentable polymers at 37 °C, indicating that the polymerization reaction is temperature-dependent.

Electron micrographic analysis of the FtsZ polymer showed formation of a dense network of FtsZ polymers in the presence of 1 M glutamate (Fig. 3, B and C), whereas only a few polymeric filamentous structures were formed in the absence of glutamate (Fig. 3, A and D). Furthermore, analysis of the electron micrographs showed that sodium glutamate not only increased the amount of polymer but also induced formation of large

**FIG. 1.** Glutamate-induced polymerization of FtsZ. A, FtsZ (6 μM) was polymerized in buffer A containing 1 mM GTP at 37 °C, in the absence (Δ) and presence of 0.25 (▲), 0.5 (○), 0.75 (□), and 1 M (●) glutamate. The rate and extent of the polymerization reaction were measured by light scattering at 500 nm using a fluorescence spectrophotometer. The light-scattering signals were corrected by subtracting appropriate blank scans. B, FtsZ (8 μM) was polymerized in buffer A containing 1 mM GTP at 37 °C for 10 min. Then 1 M glutamate was added to the cuvette, and the polymerization of FtsZ was monitored for an additional 15 min. The glutamate solution was added from a stock solution of 2 M glutamate, which reduced the concentration of FtsZ to 4 μM. The light-scattering data were corrected with respect to buffer A and 1 M glutamate.

**FIG. 2.** Determination of polymer mass by a sedimentation assay. FtsZ (6 μM) was polymerized in buffer A containing 1 mM GTP in the absence and presence of different concentrations of glutamate at 37 °C for 30 min and sedimented at 37,000 × g at 25 °C for 45 min. The protein concentrations of the supernatants were determined by the Bradford method. The protein concentrations of the pelleted fractions were determined by subtracting the supernatant concentrations from the total protein concentrations. Data were averages of three independent experiments.

**FIG. 3.** Electron micrographs of FtsZ polymers. FtsZ (6 μM) was polymerized in buffer A in the absence or presence of 1 M glutamate at 37 °C. The samples were prepared for electron microscopy as described under “Experimental Procedures.” A (×10,000) and D (×28,000) show FtsZ polymers in the absence of glutamate and B and C (×10,000) and E and F (×28,000) show FtsZ polymers formed in the presence of 1 M glutamate.
filamentous polymeric structures with extensive bundle formations (Fig. 3, E and F).

Does Glutamate Stabilize FtsZ Polymers against Dilution-induced Disassembly?—We wanted to determine whether glutamate could stabilize FtsZ polymers against dilution-induced disassembly. FtsZ (1 mg/ml) was initially polymerized with 10 mM calcium in the presence of 1 mM GTP. The polymerized FtsZ was diluted 20 times into buffer A containing different concentrations (0, 0.25, 0.5, 0.75, and 1 mM) of glutamate at 37°C. Samples were centrifuged, dissolved, and loaded on a 10% SDS-PAGE as described under “Experimental Procedures.” A, the intensities of the bands were determined by a gel documentation system from Kodak Digital Science, and relative protein concentrations of these bands were determined with respect to control. The data were plotted against glutamate concentrations (B).

Glutamate Reduces the GTPase Activity of FtsZ—FtsZ is a GTPase, and the polymerization of FtsZ is thought to be regulated by GTP hydrolysis (14, 37). Initially, we wanted to know whether GTP is essential for glutamate-induced FtsZ polymerization. The FtsZ polymerization reaction was carried out for 10 min in the presence of 1 mM glutamate (○) or 10 mM CaCl₂ (●) without GTP, and the assembly reaction was monitored for 10 min by light scattering 500 nm. After 10 min of reaction as marked by an arrow, 1 mM GTP was added to the cuvette, and the polymerization reaction was followed for an additional 10 min. B, effects of glutamate on the GTPase activity of FtsZ. FtsZ (6 μM) was mixed with different concentrations of glutamate on ice. After the addition of 1 mM GTP to the reaction mixtures, the sample solutions were immediately transferred to 37°C and incubated for 5 min. The extent of GTP hydrolysis was measured as described under “Experimental Procedures.” The data are averages of three experiments.
polymerization and promote the formation of tubulin-like protofilaments in the polymers (21, 22). Thus, it was interesting to compare the effects of glutamate and calcium on FtsZ polymerization. As previously reported, we also found that calcium induced polymerization of FtsZ, as evident from the increase in light scattering (Fig. 6A). However, glutamate (1 M) strikingly increased the magnitude of light scattering compared with 10 mM calcium (Fig. 6A). The increased light scattering by glutamate compared with calcium might be due to an increase in the polymer mass, to changes in the size and shape of the polymers, or to a combination of both the factors. To discern the possibilities, a sedimentation assay and electron microscopy were performed. We found that similar amounts of polymeric FtsZ were sedimented when the polymerization reaction was initiated by either 10 mM calcium or 1 M glutamate (Fig. 6B). Thus, the observed increase in the light-scattering intensity in the presence of glutamate was not due to an increase in the polymer mass. The electron micrographs of FtsZ polymers formed in the presence of 10 mM calcium are shown in Fig. 6C. These polymers are similar to previously reported calcium-induced FtsZ polymers (21). However, large filamentous structures containing thick bundles of FtsZ polymers were more abundant in the presence of 1 M glutamate (Fig. 3) compared with calcium (Fig. 6C). Therefore, the observed increase in light scattering in the presence of glutamate must be due to extensive bundling of FtsZ polymers.

**DISCUSSION**

In this report we found that monosodium glutamate increased the rate and extent of FtsZ polymerization. It also enhanced the bundling of FtsZ filaments, and it stabilized the polymers against dilution-induced disassembly. At low protein concentrations, FtsZ assembly reaction is believed to be isodesmic (non-cooperative). Under these conditions, FtsZ monomers assemble into single-stranded polymers with few protofilament bundles (19). However, polymers such as DEAE-dextran and calcium have been shown to modify the morphology of FtsZ polymers. In the presence of DEAE-dextran, FtsZ protofilaments associate to form three-dimensional sheets (20, 38) and millimolar concentrations of calcium-pronounced bundling of FtsZ filaments have been observed by several groups (21–23). It was suggested that DEAE-dextran and calcium increase the lateral interactions between the protofilaments (20, 39).

Hydrophobic interactions play an important role in FtsZ assembly. It was proposed previously that drugs like bis-1-anilino-8-naphthalenesulfonate inhibits FtsZ polymerization, presumably by blocking intermolecular hydrophobic interactions between FtsZ molecules (40), whereas binding of calcium or DEAE-dextran to FtsZ increases the intermolecular hydrophobic interactions, resulting in stimulation of FtsZ assembly (22, 38). In our experiments we found that the glutamate increased the rate and extent of the FtsZ polymerization to an optimal effect at a concentration of 1 M. At the optimal concentration of glutamate, FtsZ polymerization was biphasic, with a rapid burst phase of polymerization followed by a slower and prolonged linear phase (Fig. 1A). Electron micrographs of glutamate-enhanced FtsZ polymers showed large filamentous structures with extensive bundles (Fig. 3). These polymeric structures were somewhat similar to the FtsZ polymers formed in the presence of high concentrations of calcium (22); however, more bundles were present in the absence of glutamate compared with calcium. The results indicated that, like calcium, glutamate could increase the intermolecular hydrophobic interactions in FtsZ and stabilize the polymers by enhancing bundle formation. The slower phase of polymerization observed by light scattering could be due to the formation of the FtsZ polymer bundles. Furthermore, when preformed FtsZ polymer was diluted into different concentrations of glutamate, the

**TABLE I**

| Glutamate concentration (M) | Phosphate ions produced/mol of FtsZ (± S.D.) |
|-----------------------------|---------------------------------------------|
| 0.00                        | 69 ± 11                                     |
| 0.25                        | 74 ± 10                                     |
| 0.50                        | 56 ± 5                                      |
| 0.75                        | 53 ± 7                                      |
| 1.00                        | 36 ± 7                                      |

**Fig. 6.** A, FtsZ (6 μM) was polymerized in the presence of either 10 mM CaCl₂ (○) or 1 M glutamate (●). The control polymerization reaction (●) was carried out in buffer A in the absence of both CaCl₂ and glutamate. The polymerization reaction was monitored by light scattering at 500 nm. B, after 30 min of polymerization at 37 °C, FtsZ polymers were centrifuged at 50,000 × g, and pellets were dissolved in 25 mM Pipes buffer containing 0.5% SDS. The protein content of the pellets was determined by Coomassie staining of a 10% SDS-PAGE. Lanes 1 and 2 show the Coomassie-stained gel photographs of FtsZ sediments at 37 °C. The samples for electron microscopy were prepared as described under “Experimental Procedures,” and the pictures were taken at ×27,500 magnifications.
Glutamate-induced FtsZ Assembly

Glutamate polymerizes to form a dynamic ring marking the division plane of prokaryotic cells and plays an essential role in cytokinesis (1, 3). Despite its actin-like role, FtsZ has structural and biochemical similarity to tubulin (5, 11, 15, 17). Tubulin, the eukaryotic homolog of FtsZ, has been successfully targeted for the development of anticancer and antifungal drugs (47–50). Thus, the potential use of FtsZ as a drug target for the development of antimicrobial agents needs to be explored. Several potent inhibitors of tubulin assembly including colchicine and vinblastine have no effect on FtsZ assembly, suggesting that these drugs do not interact with FtsZ (40). Interestingly, calcium depolymerizes microtubules (51), whereas it promotes the polymerization of FtsZ. Furthermore, glycerol, Me$_2$SO and taxol are well characterized inducers of microtubule assembly, but these agents do not induce FtsZ assembly, suggesting that the polymerization reaction of FtsZ is mechanistically different from that of tubulin assembly. The finding of new modulators of FtsZ assembly-like glutamate would certainly help to characterize FtsZ polymerization and may help to search for new inhibitors. For example, a screen could be designed in which inhibitors of glutamate-induced polymerization might be identified.

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