Visualization of Single Escherichia coli FtsZ Filament Dynamics with Atomic Force Microscopy*

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FtsZ, the prokaryotic homologue of tubulin, is an essential cell division protein. In the cell, it localizes at the center, forming a ring that constricts during division. In vitro, it binds and hydrolyzes GTP and polymerizes in a GTP-dependent manner. We have used atomic force microscopy to study the structure and dynamics of FtsZ polymer assembly on a mica surface under buffer solution. The polymers were highly dynamic and flexible, and they continuously rearranged over the surface. End-to-end joining of filaments and depolymerization from internal zones were observed, suggesting that fragmentation and reannealing may contribute significantly to the dynamics of FtsZ assembly. The shape evolution of the restructured polymers manifested a strong inherent tendency to curve. Polymers formed in the presence of non-hydrolyzable nucleotide analogues or in the presence of GDP and AlF3 were structurally similar but showed a slower dynamic behavior. These results provide experimental evidence supporting the model of single-strand polymerization plus cyclization recently proposed to explain the hydrodynamic behavior of the polymers in solution.

FtsZ plays a prominent role in the division of most bacteria and Archaea assembling into a ring on the cytoplasmic surface of the cell membrane at the site of cell division. It is an ~40-kDa GTPase with structural homology to eukaryotic tubulin (1, 2).

The ring is a dynamic structure that has a fast subunit turnover (3, 4) and requires an active cell metabolism (5), although the exact role of the energy released after GTP hydrolysis is not known. It has been proposed that the role of GTP binding and hydrolysis by FtsZ might be to facilitate disassembly (6) because the protein (from Methanococcus jannaschii) is able to polymerize in the absence of nucleotide.

Under different conditions, FtsZ polymers can grow in vitro as straight single-stranded filaments (7), double-stranded filaments (8), and curved and circular forms (9). In the presence of GDP, the filaments are not rigid rods but rather thread-like filaments with varying degrees of curvature, whereas in the presence of GDP, a curved conformation with a defined bend between subunits is found (9). Polymerization in crowded environments induces the formation of ribbon-like structures (10).

The mechanism driving FtsZ assembly dynamics is not known. Like eukaryotic actin and tubulin, it requires energy provided by nucleotide hydrolysis (2, 11). Nevertheless, the FtsZ polymers are composed mostly of GTP-bound monomers (12, 13), so their dynamic, non-equilibrium behavior cannot be explained by mechanisms such as dynamic instability or treadmilling (2, 14). The apparent cooperativity observed during assembly is not compatible with the existence of linear single-stranded filaments (15), although both can be explained by polymer cyclization (16). Finally, it is not known whether polymer growth takes place exclusively at the ends or whether fragmentation and annealing participate in filament dynamics.

In the present work, we have used atomic force microscopy (AFM)1 to study the structure and dynamics of polymer assembly on a mica surface covered by a buffer solution. AFM provides enough spatial resolution to distinguish individual protofilaments in an aqueous environment and has allowed us to observe the polymerization and depolymerization of individual protofilaments. We show that fragmentation and annealing play a role in FtsZ polymer dynamics and that curved and circular single-stranded filaments form from GTP-bound monomers both in solution and on the mica surface. Polymers formed in the presence of non-hydrolyzable nucleotide analogues or in the presence of GDP and aluminum fluoride (AlF3) are structurally similar but showed a slower dynamic behavior, pointing toward a role of GTP hydrolysis in regulating the monomer exchange rate in the filaments. The data presented here provide experimental evidence supporting the model of single-strand polymerization plus cyclization recently proposed to explain the hydrodynamic behavior of the FtsZ polymers in solution (16).

MATERIALS AND METHODS

Reagents—Guanine nucleotides GDP and GTP were from Sigma and Roche Molecular Biochemicals, respectively. The nucleotide analogue GMPCPP, which hydrolyzes more slowly than GTP, was purchased from Jena Bioscience. Other analytical grade chemicals were from Merck or Sigma.

Protein Purification and Assay—Escherichia coli FtsZ was purified by the calcium-induced precipitation method as described previously (22). The protein concentration was measured using the BCA assay

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1 The abbreviation used is: AFM, atomic force microscopy.
GTPase activity was measured by thin layer chromatography, nucleotide exchange was measured with a nitrocellulose filter binding assay, and polymerization was followed by light scattering and electron microscopy using methods described previously (12). Formation of AlF$_3$ in solution was done by addition of NaF and AlCl$_3$ to concentrations of 10 mM and 100 mM, respectively.

**Analytical Ultracentrifugation**—Sedimentation velocity experiments were done as described previously (16) in a Beckman XL-I ultracentrifuge (Beckman Coulter) at 40,000 rpm and 20 °C. Differential sedimentation coefficient distributions, $c(s)$, were calculated by least squares boundary modeling of sedimentation velocity data using the program SEDFIT (23). The data shown in Fig. 4D, as in Ref. 16, correspond to sedimentation coefficients corrected for temperature and buffer composition.

**Atomic Force Microscopy**—Atomic force microscope images were taken with a microscope from Nonatec Electrónica (Madrid, Spain) operated in the jump mode (24). The scanning piezo was calibrated using silicon calibrating gratings (NT-MDT, Moscow, Russia). Silicon nitride tips (DI Instruments) with a force constant of 0.05 Newton/m were used. A drop of the solution with the FtsZ polymers (formed upon addition of 10 mM GTP to FtsZ protein solutions in Tris 50 mM, pH 7, 0.5 mM KCl, and 5 mM MgCl$_2$ buffer) was incubated over freshly cleaved circular pieces of mica glued onto a Teflon surface. After a few minutes, the protein solution was removed, and samples were washed extensively with working buffer and imaged under buffer solution containing 1 mM GTP. Images of the same area were acquired over a period of more than 1 h. To confirm that the imaging itself was not perturbing the dynamic behavior of the polymers, we analyzed regions away from the previously scanned surface and always observed similar changes (images not shown). The thickness of the filaments given was measured at the full width half maximum of each filament profile in order to eliminate the effect of the tip width convolution. At least three different tips were used for the measurements.

**RESULTS**

**Structure of FtsZ Filaments**—FtsZ polymerization was started in solution by adding GTP or the non-hydrolyzable analogue GMPCPP at neutral pH and high potassium concentration (see “Materials and Methods”). These conditions have been shown previously to produce maximal polymerization as measured by light scattering and maximal GTPase activity, while minimizing the degree of oligomer formation by FtsZ-GDP. The reactions were placed over freshly cleaved circular mica pieces, and after a few minutes, the reaction mixture was exchanged by buffer, and the protein adsorbed to the mica surface was observed with the atomic force microscope in a liquid environment (Fig. 1). The concentration of FtsZ was

![Image](http://www.jbc.org/)

**Fig. 1.** **Topographic image of FtsZ filaments.** FtsZ filaments formed in the presence of GMPCPP. The profile in the right panel corresponds to the line indicated in the image (left panel). **A**, the width of the filament indicated is 4.8 nm, measured at full width half maximum, and the filament height is shown to be 4 nm. **Scale bar, 100 nm.** **B**, the width of the filament is 6 nm, measured as the distance between the top of densely packed filaments. **Scale bar, 60 nm.**
Structure and Dynamics of FtsZ Polymers with AFM

Dynamics of Individual FtsZ Filaments—FtsZ filaments formed in solution in the presence of GTP were layered on mica and observed under buffer solution containing 1 mM GTP. The thickness of the filaments was measured from surfaces with a low density of filaments (Fig. 1A) in which the height was measured to be 4 nm, and on densely packed surfaces (Fig. 1B), in which the tip could not penetrate to reach the mica surface, and the width was measured as the distance between adjacent filaments. In both cases, the width measurements are only compatible with single-strand filaments forming a single layer of proteins on the mica surface.

Examination of the images provides information regarding the mechanism by which the restructuring of the filaments takes place. We observe fragmentation of some filaments that eventually reanneal, constituting a new filament (Fig. 3A); formation of filament bundles by lateral interaction of independent filaments (Fig. 3B); and cyclization of filaments (Fig. 3C).

The radius of curvature of the filaments is variable, and from the length of the circles and the monomer size, we estimate that the angle between monomers is within the range of 1° to 6°.

FtsZ Polymer Dynamics in the Presence of GDP-AlF₃—To investigate the role of nucleotide hydrolysis in the curvature and dynamics of the polymers, we used AlF₃. This compound is a high affinity analogue of the γ-phosphate of GTP at the active site and blocks the nucleotide exchange, consequently inhibiting GTPase activity of FtsZ (19). We looked at the nucleotide exchange rate and the stability and structure of the filaments when GTP was replaced by the GDP-AlF₃ complex.

We have previously shown that the nucleotide turnover of FtsZ polymers is very fast (12), reflecting not only GDP/GTP exchange after GTP hydrolysis but also that the GDP-binding site is open to the reaction solution (6, 12, 20). To study the effect of AlF₃ on the nucleotide exchange dynamics, [α-32P]GTP was added to the reaction mix and left for several minutes to obtain [α-32P]GDP-bound FtsZ, and then AlF₃ was added, the nucleotide was chased with an excess of cold GDP, and the bound nucleotide was monitored with a nitrocellulose binding assay (12). We have shown previously that most of the label is chased by an excess of unlabeled GTP in a few seconds, irrespective of the polymerization state of the protein. In the presence of AlF₃, ~70% of the label remained bound to the protein after 10 min (Fig. 4A), suggesting that the GDP-AlF₃ has high affinity for the nucleotide-binding site and blocks the nucleotide exchange, consequently inhibiting GTP hydrolysis.

The polymerization of FtsZ in the presence of AlF₃ was studied by light scattering (Fig. 4B). The protein was incubated at 25 °C with fresh GDP in polymerization buffer. Addition of AlF₃ induced a rapid polymerization that remained stable for at least 30 min. The same finding was observed when the protein was incubated with GTP, and the AlF₃ was added after the protein had polymerized and depolymerized completely. Polymerization was not induced by AlF₃ in the absence of nucleotide, and neither NaF nor AlCl₃ alone induced polymerization, indicating that the complex AlF₃ binds to FtsZ-GDP and that the bound GDP-AlF₃ is a high affinity analogue of GTP that induces polymerization but cannot be exchanged, and therefore the polymers formed are stable.

The apparent critical concentration of the polymerization reaction in the presence of GDP and AlF₃ was calculated from the light scattering signal obtained at different FtsZ concentrations (Fig. 4C). The critical concentration obtained was 2 μM,
which is not very different from the concentrations measured in previous works (~1 μM) (10), indicating that AlF₃ binding does not alter the affinity between protein subunits.

We have previously found by sedimentation velocity that in the presence of GTP (and a GTP regenerating system), FtsZ polymers sediment as a sharp peak that indicates that they have a size limit and are relatively homogeneous (16). Similar sedimentation velocity behavior has been found in the case of FtsZ polymers induced by binding of GDP and AlF₃. At low FtsZ concentrations, several peaks were seen, ranging from low to high sedimentation coefficients, whereas at higher concentrations, most of the protein concentrates into a single sharp peak with a sedimentation coefficient comparable to the fast-sedimenting peak previously observed in the presence of GTP (Fig. 4D).

**Curvature of FtsZ Filaments in the Presence of GDP-AlF₃**—The polymers formed in the presence of AlF₃ were studied by electron microscopy and AFM (Fig. 5). Electron micrographs showed that the polymers were thin, apparently single-stranded filaments 5.6 ± 0.1 nm wide (n = 51) that formed circular and curved structures similar to those observed in GTP at low protein concentrations (16). AFM images also showed curved and circular structures, with a tendency to interact laterally, indistinguishable from the structures formed in the presence of GTP. The difference, however, was observed in their higher stability because the structures remained rela-
tively stable for up to 30 min (Fig. 5B), as compared with the highly dynamic behavior observed in the GTP polymers shown in Fig. 2.

DISCUSSION

The atomic force microscope has allowed us to visualize and follow the growth and depolymerization of individual FtsZ protofilaments on a mica surface kept under buffer solution. We have obtained information about two functionally relevant traits of FtsZ polymers: 1) the assembly/disassembly processes and 2) their tendency to form curved structures.

Dynamic Restructuring and Flexibility—Filaments polymerized in solution and adsorbed onto mica covered the surface with a network of continuous individual and highly flexible filaments. A strong tendency to align laterally and to aggregate into transient bundles was observed (Fig. 1), suggesting the presence of lateral interactions. The 4-nm filament height measured corresponds well with the crystal structure dimensions (1), indicating that under our imaging conditions, the interaction with the mica and the imaging tip did not significantly deform the protein (Fig. 1).

GTP consumption was accompanied by a continuous restructuring of the filaments. The observed behavior is better explained considering the filaments as linear polymers of interacting monomers, all having the potential to dissociate from neighboring monomers. There are two distinctive characteristics of the FtsZ polymers that could explain why this mechanism is energetically possible: 1) FtsZ filaments are single-stranded, and 2) single-stranded FtsZ polymers consist largely of GTP-bound subunits (12, 13). For single-stranded filaments, the energetic difference between dissociating a monomer from the end and dissociating it from the interior is the difference between breaking one or two energetically equivalent bonds. For double- or multi-stranded filaments, this difference is larger, given the multiple interactions of the internal monomers. For single-stranded filaments, the energetic difference between dissociating a monomer from the end and dissociating it from the interior is the difference between breaking one or two energetically equivalent bonds. For double- or multi-stranded filaments, this difference is larger, given the multiple interactions of the internal monomers. For single-stranded filaments, the balance between GTP hydrolysis and rapid GDP/GTP exchange along the filament (12, 13, 21) could produce transient and labile monomer interactions that could make inner monomer dissociation as energetically feasible as dissociation of end monomers. Fig. 3A points out individual episodes of protofilament rearrangement
showing fragmentation due to dissociation between internal monomers and reannealing with adjacent filaments providing experimental support for this interpretation. The highly dynamic and plastic behavior observed in the filaments is therefore explained through a versatile and flexible mechanism for polymerization allowed for in single-stranded filaments capable of fragmenting at any point and reannealing with adjacent filaments.

Fragmentation of polymer filaments might be important to determine polymer dynamics either in solution or on a surface. In contrast, reannealing with other filaments is more likely to be relevant for surface dynamics, whereas in solution, self-annaling inducing cyclization might be favored. The more static behavior of filaments formed in the presence of GDP-AlF₃ suggests that the energy provided by nucleotide hydrolysis is relevant in maintaining the dynamism underlying the observed structural plasticity of the filaments, as has been suggested previously (6).

Polymer Curvature—The second issue addressed in this work is the nucleotide dependence of the filament curvature. We observe curved filaments in all conditions explored: 1) in the presence of an excess of GTP (where, given the biochemical evidence that most monomers in the polymer are GTP-bound, we can assume that at any moment only a small number of monomers have GDP); 2) in the presence of GMPCPP (low hydrolyzing nucleotide; therefore, a low rate of GDP formation and presumably a low GDP-monomer concentration); the curvatures observed in both of the above conditions are smooth and not kinked, as would be expected if the curvature was only induced at the points of insertion of GDP monomers in the filaments; and 3) in the presence of AlF₃. The GDP-AlF₃ complex is a high affinity (non-exchangeable) analogue of GDP-Pᵢ that is anchored in the GTP-binding site. We can therefore assume that under these conditions, there are no GDP-bound monomers present in the filaments and that the curved filaments observed are constituted by monomers that mimic the GTP-bound conformation.

The range of angles between monomers we observed is different from the larger angle needed for the formation of previously described mini-rings of about 25 nm in diameter (9). The curved structures we observe are, however, well within the range of diameters compatible with the cellular dimensions during the constriction that leads to cell division.

The operation of lateral interactions between existing protofilaments can also be inferred from our results as being relevant for determining the higher order structures of the polymers (Fig. 3B). We had previously reported that the presence of crowding agents to mimic the high excluded volume present in the interior of the living cell induced higher order polymer structures that are compatible with the existence of these lateral interactions (10).

Until now, the existence of single-stranded filaments had been difficult to reconcile with the cooperative polymerization behavior observed in vitro (15). However, recent experimental evidence from analytical centrifugation has led us to propose an isodesmic polymerization plus cyclization model that explains the cooperative behavior earlier attributed only to the formation of double-stranded filaments (16). The highly flexible and circular single-stranded filaments formed in the presence of GTP or GDP-AlF₃ described in this work provide further experimental support to the proposed polymerization model.

In summary, we have observed that curved single-stranded filaments form in the presence of GTP at neutral pH and high ionic strength, supporting the recently proposed isodesmic cyclization model. We have also provided direct evidence that fragmentation and reannealing, two processes that affect the assembly kinetics, occur in FtsZ filaments on a surface. This behavior might be relevant for the dynamic assembly of the FtsZ ring on the inner cell membrane during bacterial cell division.

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