The Cell Division Protein FtsZ from *Streptococcus pneumoniae* Exhibits a GTPase Activity Delay*

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**Background:** The FtsZ cell division protein has nucleotide-dependent GTPase and assembly activities. The mechanism coupling these two activities is uncertain.

**Results:** Purified *Streptococcus pneumoniae* FtsZ (SpnFtsZ) presents a lag in the GTPase activity but not in filament assembly.

**Conclusion:** The differences between the initial polymerization and GTPase activities of SpnFtsZ suggest a transition from inactive to active polymers.

**Significance:** Nucleotide hydrolysis by SpnFtsZ polymers involves an activation step.

The cell division protein FtsZ assembles *in vitro* by a mechanism of cooperative association dependent on GTP, monovalent cations, and Mg$^{2+}$. We have analyzed the GTPase activity and assembly dynamics of *Streptococcus pneumoniae* FtsZ (SpnFtsZ). SpnFtsZ assembled in an apparently cooperative process, with a higher critical concentration than values reported for other FtsZ proteins. It sedimented in the presence of GTP as a monodisperse species, depending on the experimental conditions (16–19).

Some models of cooperativity involve a nucleation step followed by protein conformational changes that would increase the affinity of the next monomer to be bound to the growing polymer with an average mass of 100 ± 20 FtsZ monomers (19, 24). Some models of cooperativity involve a nucleation step followed by protein conformational changes that would increase the affinity of the next monomer to be bound to the growing polymer (23, 25). In this way, the polymerization of *Mycobacterium tuberculosis* FtsZ (MtbFtsZ) is also described as a cooperative assembly mechanism that involves a dimer nucleus, similar to EcFtsZ, but with kinetics ~10 times slower (21). No structural evidence of nucleotide-dependent conformational changes has been found (26), although some have been proposed on the basis of analyses of tryptophan mutants and exchanges FtsZ monomers with a turnover of a few seconds (9).

**Conclusion:** The proto-ring recruits the remaining essential division proteins to complete the divisome, the functional division machinery that exerts the force needed for cell constriction and conducts the biosynthesis of the septal cell wall (10–12). Because of its unique polymerization dynamics, FtsZ is also widely considered to have an active role in generating the motor force needed to pull the membrane and cell envelope inwards during cell division (13).

**Escherichia coli** FtsZ (EcFtsZ), the most studied FtsZ protein, associates in the presence of GDP and Mg$^{2+}$ following an isodesmic model to form short oligomers (14, 15). In the presence of GTP, Mg$^{2+}$ and a monovalent cation (K$^+$, Na$^+$), EcFtsZ assembles into long polymers of different lengths and structures, depending on the experimental conditions (16–19). Polymerization is very fast after GTP addition (20), rendering the kinetics of protein assembly particularly difficult to study.

The existence of a critical protein concentration for assembly suggests a cooperative polymerization mechanism, but the details of the mechanism are not known (21–23). The dependence of protein assembly on Mg$^{2+}$ concentration has been analyzed in the presence of constantly replenished GTP in steady state conditions using sedimentation velocity, concentration-gradient light scattering, fluorescence correlation spectroscopy, and dynamic light scattering, confirming the cooperative assembly of a narrow size distribution of fibrillar polymers with an average mass of 100 ± 20 FtsZ monomers (19, 24).

FtsZ is the main cytoskeletal protein involved in the division of most prokaryotes. The protein is generally considered the ancestor of tubulin, because it is a GTPase that polymerizes in a GTP-dependent manner (1), and they have a significant three-dimensional structure similarity (2, 3). Cell division begins with FtsZ localization and membrane anchoring, via other essential division proteins like FtsA, to form an annular structure at the division site termed the proto-ring (4–7).

The ring is a dynamic structure that requires a constant energy input (8) and...
molecular dynamics studies (27–29). An alternative model proposed an isodesmic growth cyclization process that would result in cooperativity (18, 30).

The *Streptococcus pneumoniae* FtsZ (SpnFtsZ) has not been characterized at the biochemical or biophysical level before. The protein was initially identified by Western blot analysis in *Streptococcus* cell extracts (31). More recently, FtsZ was quantified (3000 molecules/cell) and was shown to be essential for division (32); in immunostaining, it co-localized in cells with FtsA to form the midcell ring (33). Here we purified SpnFtsZ and analyzed its biochemical and functional properties, with emphasis on polymerization and GTPase activity, and compared them with EcFtsZ.

**Experimental Procedures**

*SpnFtsZ Gene Cloning*—Standard protocols for molecular cloning, transformation, and DNA analysis were as described (34). Plasmids encoding the streptococcal FtsZ protein for overproduction and purification were obtained by subcloning the ftsZ gene from the pBAD-FtsZ vector (kindly provided by Dr. Orietta Massidda, University of Cagliari, Cagliari, Italy). Primers MK14 (5′-GGGATCCCATATGACATTTTCATTTGATACAGC-3′) and MK15 (5′-CGGAATTCCTTAACGA-TTTTGGAAAAATGG-3′) (Sigma-Aldrich) were used to amplify the ftsZ gene sequence. This gene was inserted into the Ndel-EcoRI restriction sites of the overexpression vector pET24a (Invitrogen), yielding the plasmid pMKV18, which encodes the SpnFtsZ protein without the His tag. Correct gene cloning was confirmed by DNA sequencing.

*SpnFtsZ Purification*—To purify SpnFtsZ, *E. coli* C41 cells transformed with plasmid pMKV18 were grown (2 h, 37 °C) in 2 liters of Luria broth supplemented with kanamycin (50 μg ml⁻¹). At an *A*₃₅₀ of 0.45, SpnFtsZ overproduction was induced for 3 h by addition of 1 mM isopropyl-1-thio-β-β-galactopyranoside. Cells were harvested by centrifugation (5,000 × g, 20 min, 4 °C), resuspended in 40 ml of PEM buffer (50 mM PIPES, pH 6.5, 1 mM EDTA, 5 mM MgCl₂), and frozen at −80 °C. Cells were lysed by sonication, and the soluble fraction containing SpnFtsZ was purified by Ca²⁺-induced precipitation (15). Briefly, the lysate was cleared by centrifugation (50,000 × g, 30 min at 4 °C), and then GTP and CaCl₂ were added to the supernatant to 1 and 20 mM final concentrations, respectively. The mixture was incubated 15 min at 30 °C and centrifuged (30,000 × g, 15 min at 4 °C). The pellet containing FtsZ was resuspended again in PEM buffer, cleared by centrifugation (30,000 × g, 15 min at 4 °C), and the supernatant was subjected to another cycle with GTP and CaCl₂. The pelleted protein was resuspended in 50 mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 5 mM MgCl₂, and loaded into a HiTrap Q FF column in an ÄKTAprime plus system (GE Healthcare) equilibrated in the same buffer. The column was washed with the equilibration buffer and next with a 0–1 M gradient of KCl in buffer. The protein eluted in a range of 300–400 mM KCl, fractions were pooled and dialyzed against 50 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 300 mM KCl. Protein purity was confirmed by SDS-PAGE.

*Protein Identification by Peptide Footprint*—As SpnFtsZ showed a higher relative molecular mass than predicted, and the protein sequence was confirmed by mass spectrometry analysis of peptides produced by proteolysis. Trypsin-digested samples were analyzed with an Autoflex III TOF/TOF mass spectrometer (Bruker-Daltonics). MALDI-MS and MS/MS data were combined through the BioTools 3.0 program (Bruker-Daltonics), and Mascot software 2.3 (Matrix Science) was used for amino acid sequence analysis with NCBI nonredundant protein database (accession number 20100306). Protein scores >86 were considered as being significant (*p* < 0.05) (35).

* Determination of Protein Concentration*—Because EcFtsZ purified by the same method as SpnFtsZ bears a GDP molecule and protein absorbance at 280 nm is affected by the nucleotide (15), we calculated protein concentration using the Bradford colorimetric assay (Bio-Rad) and BSA as reference (SpnFtsZ and BSA produced identical color in the colorimetric assay). This method reproducibly estimates SpnFtsZ concentration, as confirmed by quantitative amino acid analysis (36). GDP content was estimated by protein precipitation with 10% (v/v) perchloric acid as described (15).

* Analysis of FtsZ Polymerization by Static Light Scattering*—SpnFtsZ or EcFtsZ proteins were diluted to desired concentrations in polymerization buffer (50 mM Tris-HCl, pH 7.5, 250 mM KCl, 5 mM MgCl₂). Five seconds after addition of 1 mM GDP or GTP, FtsZ polymer formation was monitored by 90° light scattering measurement at 24 °C on a Hitachi F-2500 fluorescence spectrophotometer. Excitation and emission wavelengths were set to 320 nm with 5-nm slit widths, as described (20).

*Electron Microscopy*—To observe SpnFtsZ polymer morphology, the polymerization reaction described above was terminated at 4 min, and samples were adsorbed on a 400-mesh collodion-coated, glow-discharged copper grid for 1 min, negatively stained with 2% (w/v) uranyl acetate, then imaged with a Jeol JEM1011 transmission electron microscope, and photographed with an 11 megapixel charge-coupled device camera (Gatan Etlangshen ES1000W). Images were captured with Gatan Digital Micrograph 1.8.0 software and processed with Adobe Photoshop CS5. FtsZ polymer length and width were measured using the public image analysis program ImageJ. Approximately 100 polymers were analyzed for length, and 50 paired filaments were analyzed for width.

*Analytical Centrifugation*—Sedimentation velocity experiments were performed in an Optima XL-I ultracentrifuge (Beckman Coulter) equipped with absorbance and interference optics to monitor SpnFtsZ sedimentation in the presence of 1 mM nucleotide (GDP or GTP). Various concentrations of SpnFtsZ were equilibrated in polymerization buffer and immediately before sedimentation, and SpnFtsZ samples were supplemented with 1 mM GDP or GTP and centrifuged (30,000–48,000 rpm, 20 °C) in an AN50Ti eight-hole rotor and double-sector Epon-charcoal centrepieces. For polymer formation with GTP, a GTP regeneration system (GTP/RS, containing acetate kinase and acetyl phosphate) was added to avoid complete GTP depletion during data collection in sedimentation velocity analysis (37). Elapsed time between GTP/RS addition and the last scan used in data analysis was adjusted for optimal visualization of GTP-SpnFtsZ polymers in solution (polymers were stable for ~50 min). Differential sedimentation coeffi-
cient distribution $c(s)$ was calculated by least squares boundary modeling of the experimental data using the SEDFIT program (38). The $s$ values were corrected to standard conditions using SEDNTERP software, yielding standard $s$ values ($s_{20,w}$).

To determine the oligomerization state of $SpnFtsZ$ in the presence of GDP, short column (85 μl) sedimentation equilibrium of the $SpnFtsZ$ samples, alone or with 0.1 mM GDP, was performed at multiple speeds (9,000, 13,000, and 18,000 rpm). Following equilibrium scans, high speed centrifugation analysis (48,000 rpm) was used to estimate the baseline offsets. Weight average buoyant molecular weight was determined by fitting a single species model to the experimental data using the HETEROANALYSIS program (39).

**Dynamic Light Scattering**—Dynamic light scattering experiments were carried out in a Protein Solutions DynaPro MS/X instrument (Protein Solutions) at 20 °C, as described (19). The data were analyzed using MATLAB (version 7.0.4; MathWorks). Correlation functions for mono- and polydisperse particles were analyzed to calculate translation diffusion coefficient values, $D_t$ (19). The apparent molar mass of polymers was calculated according to the Svedberg equation using the average of the standard translation diffusion coefficient values $D_t$ obtained by dynamic light scattering and the average $s_{20,w}$ ($s$ values corrected to standard conditions of water, 20 °C, and infinite dilution) obtained from analysis of independent sedimentation velocity profiles (24).

**GTPase Activity**—FtsZ GTPase activity was determined by the malachite green method (40). Hydrolysis reactions were performed as described above in polymerization buffer at 24 °C with 1 mM GTP. Samples were collected at various times and diluted in the same buffer containing 65 mM EDTA to terminate the reaction. Malachite green-molybdate reagent was added, and absorbance was measured at 620 nm. Phosphate concentrations were calculated from a Na₂HPO₄ standard curve, and the GTPase activity reaction rate ($V$, molP/molFtsZ/min) was determined from the slope of the linear part of phos-

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**FIGURE 1. $SpnFtsZ$ polymerization.** A and B, polymer formation in polymerization buffer (50 mM Tris-HCl, pH 7.5, 250 mM KCl, 5 mM MgCl₂) was followed by 90° light scattering after addition of 1 mM GTP. Curves (black to light gray) correspond to 5, 10, 15, and 20 μM, respectively, as indicated, for $SpnFtsZ$ (A) and $EcFtsZ$ (B). Times to maximum assembly after GTP addition were calculated from $SpnFtsZ$ profiles: 3.1 min for 10 μM ($I_{max} = 560$ AU), 2.0 min for 15 μM (1687 AU), and 1.3 min for 20 μM (3025 AU). C, polymers obtained in 4 min with 10 μM $SpnFtsZ$, as described under “Experimental Procedures,” were analyzed by electron microscopy. D, amplified image of a polymer. E–G show polymers obtained at 20, 35, and 170 s, respectively (included 20 s of adsorption time), with 15 μM $SpnFtsZ$. 

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phate accumulation curves. To determine the activation delay of the GTP hydrolysis, the curves were divided in two sections, and linear regression was done independently for each section. The delay of the GTP hydrolysis was calculated from the crossing point between the two regression lines. Enzymatic parameters (K_m and V_max) were calculated from the kinetic curves using GraphPad Prism 5.0. All reactions were performed in duplicate.

Results and Discussion

In this study we have purified and characterized the wild-type FtsZ protein from S. pneumoniae (SpnFtsZ) and compared its biochemical and assembly properties with those of E. coli (EcFtsZ), the best studied FtsZ protein.

Functional Properties of Purified SpnFtsZ—SpnFtsZ was overproduced in E. coli cells and purified at high yield (40 ± 10 mg of protein per liter of culture) in native conditions using cycles of Ca^{2+}-induced protein precipitation as previously described for EcFtsZ purification (15). Electrophoretic analysis of the purified protein showed a single band corresponding to a relative mass of 49,490 ± 900 Da (data not shown), a value slightly higher than that predicted from the amino acid sequence (44,416 Da), that lead to confirm by peptide mass fingerprinting that the protein sequence of the purified protein was the one expected. Purified SpnFtsZ has 0.7 ± 0.1 mol of bound GDP per mol of protein monomer.

SpnFtsZ polymerization was triggered with GTP, in 250 mM KCl, 5 mM MgCl_2 in 50 mM Tris-HCl, pH 7.4 (polymerization buffer) and was monitored at 24 °C by light scattering (Fig. 1A). The signal caused by polymer formation increased over 1–3 min before reaching the maximum level of polymerization. The reaction was faster for the highest protein concentration assayed (1 min for 20 μM SpnFtsZ, 3 min for 10 μM). Polymers disassembled after a few minutes. No assembly was observed at 5 μM SpnFtsZ. On the other hand, the polymers of EcFtsZ monitored in conditions identical to those of SpnFtsZ reached their maximum polymerization signal immediately at any protein concentration assayed (5–20 μM EcFtsZ) (Fig. 1B).

The SpnFtsZ polymers consisted of single- and double-stranded filaments, the latter being predominant, with a narrow size distribution (10 ± 1 nm, 540 ± 200 nm) as revealed by negative stain electron microscopy (Fig. 1, C and D). There are no qualitative differences in the structure of the polymers at the beginning of the reaction (Fig. 1, E–G) as shown in the images obtained at shorter times (20, 35, and 170 s respectively). Under similar polymerization conditions, EcFtsZ preparations showed individual filaments and up to 3–4 lateral associated filaments, as reported (1, 23, 41, 42). Preferentially double filaments were also observed in M. tuberculosis FtsZ (MtFtsZ) preparations at pH 6.5 (not at pH 7.7) (21) and in the thermophilic Methanococcus jannaschii FtsZ (MjFtsZ) at pH 6.5 at 55 °C (43).

The GDP Form of SpnFtsZ Does Not Oligomerize in the Presence of Magnesium—The association state of the GDP form of purified SpnFtsZ, and its possible modulation by Mg^{2+} and protein levels, as in the case of EcFtsZ (15, 24), were measured by analytical ultracentrifugation. In sedimentation velocity experiments, at the highest protein concentration tested (25 μM), SpnFtsZ sediments as a main single homogeneous species, corresponding to 80–95% of total protein, with a sedimentation coefficient value of 2.9 ± 0.1 S in the presence of 1 mM GDP, independently of Mg^{2+} concentration (from EDTA to 5 mM MgCl_2) (Fig. 2A). This species is compatible with SpnFtsZ protein monomers, as confirmed by the sedimentation equilibrium analysis (Fig. 2B). From these experiments, we conclude that the GDP form of SpnFtsZ is a monomer with an overall structure that slightly deviates from the globular shape (frictional ratio = 1.5–1.6) which, unlike EcFtsZ (15), does not have a tendency to form oligomers in the presence of Mg^{2+}.

The GTP Polymer of SpnFtsZ Is Larger Than That of EcFtsZ—SpnFtsZ polymerization driven by GTP was analyzed at steady state by sedimentation velocity in the presence of a GTP-regen-
Operating system as previously done with the FtsZ protein from E. coli (18, 30, 44). In accordance with the light scattering measurements, no assembly of SpnFtsZ was detected at low protein concentration (6 μM), and the protein was distributed as slowly sedimenting species with a s value corresponding to the monomeric species (~3.0 S) (Fig. 3). At higher concentrations, most of the protein was found as a rapidly sedimenting fraction with an average sedimentation coefficient of 34 ± 3 S (s_{20,w}). This fraction represents a narrow sized distribution, resembling thus the sedimentation behavior of EcFtsZ (18, 30, 44). However, the s value of the rapid sedimenting species of SpnFtsZ is much higher than the one of the EcFtsZ polymeric species, suggesting that the average size of the protofilaments of SpnFtsZ is higher as well (18). Dynamic light scattering was then used to measure the diffusion coefficient of the rapid sedimenting species of SpnFtsZ (2.1 ± 0.1 μm^2 s^{-1}). From the measured s and D values, following the Svedberg equation, the average molar mass of the polymeric species was found to be 320 ± 25 monomers per protofilament, three times higher than the number of FtsZ subunits forming the protofilament in E. coli (100 ± 20 monomers) under similar experimental conditions (30, 44).

**FIGURE 3.** Sedimentation velocity analysis of SpnFtsZ polymer. Sedimentation coefficient distributions of three concentrations of SpnFtsZ in polymerization buffer supplemented with 1 mM GTP, in a GTP regeneration system.

**FIGURE 4.** GTPase activity of SpnFtsZ. Protein samples were incubated with 1 mM GTP in polymerization buffer. A and B, phosphate accumulation curves produced by SpnFtsZ (A) and EcFtsZ (B) were determined by duplicates at various protein concentrations (squares, 5 μM; triangles, 10 μM; crosses, 15 μM; circles, 20 μM). Error bars are drawn. C, GTPase activity delay, calculated as the time corresponding to the intersection value of the two regression lines determined in the phosphate accumulation curves obtained for a SpnFtsZ concentration, semi-log plotted against protein concentrations (p < 0.001, n = 26 independent experiments). D, increasing GTP amounts were incubated with 15 μM SpnFtsZ in polymerization buffer. Velocities were obtained from the slopes of the phosphate accumulation curves and fitted to a Michaelis-Menten model with V_{max} = 3.3 nmol GTP/nmol FtsZ/min and K_{m} = 430 μM (n = 20 independent experiments).
GTPase Activity of SpnFtsZ: an Activation Delay of the GTP Hydrolysis—At 5 μM SpnFtsZ, the protein did not hydrolyze GTP, in agreement with the absence of polymerization (Fig. 4A). At the threshold concentration for assembly (10 μM), the GTPase activity had a lag (1.5 min), during which there was polymerization but no phosphate accumulation. Then GTPase activity increased to a rate of 2.43 ± 0.02 molP/molSpnFtsZ/min. The activation delay of the GTP hydrolysis (lag plus transition phase) was strongly dependent on protein concentration, being shorter as SpnFtsZ concentration increased. The delays to attain the GTPase reaction rate calculated for 10, 15, and 20 μM SpnFtsZ were 2.4, 1.6, and 1.0 min, respectively (Fig. 4C). Reaction rates were similar at 15 μM SpnFtsZ (3.80 ± 0.42 molP/molSpnFtsZ/min) and 20 μM SpnFtsZ (3.60 ± 0.07 at 20 μM).

This behavior differed from that of EcFtsZ, for which no activation delay of the GTP hydrolysis was detected in same experimental conditions, even approximately at its critical assembly concentration (Fig. 4B). In addition, EcFtsZ GTPase activity reached its maximal rate at 5 μM protein (V = 6.76 ± 0.16 molP/molEcFtsZ/min), with a progressive decrease at higher concentrations (3.99 ± 0.06 at 10 μM and 2.26 ± 0.16 at 20 μM), in accordance with our previous reported data (45). An activation delay of the GTP hydrolysis has been described for Staphylococcus aureus FtsZ (SaFtsZ) that hydrolyzes GTP above 4 μM protein after a transition phase that was shorter as the protein concentrations increased (46).

GTPase Kinetics of SpnFtsZ—To evaluate SpnFtsZ GTPase activity kinetics, different GTP concentrations were hydrolyzed with a saturated amount of SpnFtsZ (15 μM) in polymerization buffer. Velocities of the GTP hydrolysis fitted to a Michaelis-Menten kinetic model (Fig. 4D), with V_max = 3.3 ± 0.4 nmolP/nmolFtsZ/min, and K_m = 430 ± 138 μM. Velocity of GTP hydrolysis was 2-fold lower than that reported for EcFtsZ, calculated in the same experimental conditions (V_max = 6 nmolP/nmolFtsZ/min, K_m = 300 μM) (47). On the other hand, MtFtsZ kinetic was described 10-fold slower than EcFtsZ (at pH 7.7, room temperature) (21) as well as SaFtsZ, with a lower GTP hydrolysis rate (~1.0 min⁻¹ at pH 6.5, 30 °C) (46), whereas MjFtsZ showed similar kinetic values as the E. coli protein (~5.9 min⁻¹ at pH 6.5, 55 °C) at its optimal conditions (43).

Cation Dependence of the GTPase Activity of SpnFtsZ—The GTPase activity of FtsZ from E. coli and other species depends on cation binding (48). We assayed SpnFtsZ GTP hydrolysis at different K⁺, Mg²⁺, and Na⁺ concentrations and compared with EcFtsZ values in the same conditions (1 mM GTP and standard salt concentrations except that tested). SpnFtsZ required at least 100 mM KCl to start to hydrolyze GTP, and the reaction rate reached a maximum at 200 mM KCl (Fig. 5A). In contrast, EcFtsZ reached a maximum at 100 mM KCl, which was maintained at higher K⁺ concentrations. SpnFtsZ did not hydrolyze GTP at 1 mM MgCl₂ and needed 3 mM MgCl₂ for optimal activity; EcFtsZ showed 80% of its maximum GTPase rate at 1 mM MgCl₂ (Fig. 5B). GTP hydrolysis by SpnFtsZ decreased when increasing amounts of NaCl were present in the GTPase reaction, and it was completely inhibited at 250 mM NaCl (Fig. 5C). EcFtsZ activity showed no GTPase inhibition, even at 500 mM NaCl. In summary, Spn-
FtsZ requires higher K\(^+\) and Mg\(^{2+}\) concentrations than EcFtsZ to reach maximal GTPase velocity values and Na\(^+\) inhibited this activity. The specificity for K\(^+\) was also found in the GTP hydrolysis by MjFtsZ (43, 49). When Na\(^+\) is present, it competes with K\(^+\) to occupy the catalytic GTPase active site and thus inhibits protein polymerization and GTP hydrolysis (48). Na\(^+\) also inhibited SpnFtsZ polymerization detected in the light scattering assay (data not shown).

Critical Concentration of SpnFtsZ—To calculate the critical concentration of SpnFtsZ GTPase activity, velocities of GTP hydrolysis obtained in several experiments were plotted against protein concentrations (Fig. 6). Regression fitting indicated a critical SpnFtsZ concentration of 8 \(\mu\text{M}\) to hydrolyze GTP. In accordance, the degree of polymerization reached by different SpnFtsZ concentrations (10, 15, and 20 \(\mu\text{M}\)) analyzed by 90° light scattering assay (Fig. 1A) yielded the same critical concentration for SpnFtsZ assembly (Fig. 6, inset). Protein concentration dependence analyzed by sedimentation velocity supported these critical concentration calculations, because below 6 \(\mu\text{M}\) concentration no fast sedimenting species corresponding to FtsZ polymers were found, and most of the protein sediments as a polymer above 13 \(\mu\text{M}\) (Fig. 3).

The critical concentration of SpnFtsZ observed in GTPase activity/polymerization assays is higher than values reported for other FtsZ proteins: 1 \(\mu\text{M}\) EcFtsZ (14), 2 \(\mu\text{M}\) for MjFtsZ (at pH 6.5) (43), 3 \(\mu\text{M}\) for MtFtsZ (at pH 7.7) (50), and 4 \(\mu\text{M}\) for SaFtsZ (at pH 6.5) (46).

**Conclusion**

We report the biochemical and biophysical characterization of the FtsZ from *S. pneumoniae*. The critical concentration, the delay observed in the GTPase activity, coupled to progressive elongation of the polymer, both strongly dependent on protein concentration, the well defined polymer size, and the tendency to form double-stranded filaments suggested a cooperative SpnFtsZ assembly mechanism. Our results for SpnFtsZ polymerization/GTPase activity provide evidence of the theoretical “intermediate” species in FtsZ polymerization dynamics suggested in different models of FtsZ polymerization that propose a FtsZ monomer nucleation step with conformational changes in the protein (21, 23, 25, 51). In the first minute after GTP addition (Fig. 7), SpnFtsZ scattered light, indicating the presence of polymers, but we detected no GTPase activity at 10 \(\mu\text{M}\) protein (at approximately the critical concentration). This effect on polymer development has not been reported for the *E. coli* protein. The slower kinetics and the higher critical concentration of SpnFtsZ allowed us to study the initial FtsZ polymerization/GTP hydrolysis reaction and to distinguish these two functions in the same polymer sample. Polymers shaped in the first minute of the reaction were large enough to produce strong light scattering but were unable to hydrolyze GTP detectably.
Assembly and GTPase Activity of S. pneumoniae FtsZ

Although FtsZ polymers lacking GTPase activity have been obtained, including the FtsZW319Y-His6 mutant of MjFtsZ (43), McFtsZ polymers at pH 6.5 (21), or EcFtsZ polymers at pH 5.5 (48), no measurable transition of an inactive to an active polymer has yet been reported, although cooperative conformational transitions have been suggested in several models of FtsZ polymerization (25). Our data suggest that SpnFtsZ assembles through a cooperative mechanism that involves a nucleation step and a transition of the polymer from a GTPase inactive to active state.

Author Contributions—E. S., G. R., J. M., C. A., and A. I. R. designed the study and wrote the paper. E. S., M. K., P. G.-P., C. A., and A. I. R. performed research. All the authors analyzed the results and approved the final version of the manuscript.

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