Ruthenium Red-induced Bundling of Bacterial Cell Division Protein, FtsZ*

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The assembly of FtsZ plays a major role in bacterial cell division, and it is thought that the assembly dynamics of FtsZ is a finely regulated process. Here, we show that ruthenium red is able to modulate FtsZ assembly in vitro. In contrast to the inhibitory effects of ruthenium red on microtubule polymerization, we found that a substoichiometric concentration of ruthenium red strongly increased the light-scattering signal of FtsZ assembly. Further, sedimentable polymer mass was increased by 1.5- and 2-fold in the presence of 2 and 10 μM ruthenium red, respectively. In addition, ruthenium red strongly reduced the GTPase activity and prevented dilution-induced disassembly of FtsZ polymers. Electron microscopic analysis showed that 4–10 μM of ruthenium red produced thick bundles of FtsZ polymers. The significant increase in the light-scattering signal of sedimentable polymer mass in the presence of ruthenium red seemed to be due to the bundling of FtsZ protofilaments into larger polymers rather than the actual increase in the level of polymeric FtsZ. Furthermore, ruthenium red was found to copolymerize with FtsZ, and the copolymerization of substoichiometric amounts of ruthenium red with FtsZ polymers promoted cooperative assembly of FtsZ that produced large bundles. Calcium inhibited the binding of ruthenium red to FtsZ. However, a concentration of calcium 1000-fold higher than that of ruthenium red was required to produce similar effects on FtsZ assembly. Ruthenium red strongly modulated FtsZ polymerization, suggesting the presence of an important regulatory site on FtsZ and suggesting that a natural ligand, which mimics the action of ruthenium red, may regulate the assembly of FtsZ in bacteria.

FtsZ has a limited sequence homology with tubulin, it shares a common GTP binding motif with tubulin, it assembles into dynamic protofilaments in a GTP-dependent manner, and it has significant structural similarities with tubulin, suggesting that FtsZ is a prokaryotic homolog of tubulin (8–15). Purified FtsZ monomers assemble into single-stranded protofilaments with little or no bundling of protofilaments in a polymerization reaction that is believed to be isodimic in nature (16). Several factors are known to affect FtsZ assembly dynamics and bundling of protofilaments in vitro (17–22). For example, DEAE-dextran and polycation lipid monolayer are shown to induce protofilament association into sheets (17). Recently, divalent calcium (1–10 mM) and monosodium glutamate (0.1–1 mM) were found to stabilize polymers and induce bundling of protofilaments (18, 19). In addition, a neutral macromolecule Ficoll (200 g/liter) was found to induce bundling of FtsZ protofilaments into ribbon-like structures (23). Further, a basic protein ZipA induces bundling of FtsZ polymers both in vitro and in Escherichia coli (24–26). The bundling of FtsZ protofilaments is thought to play an important role in the functioning of the cytokinetic Z-ring during bacterial division (27, 28).

The known inducers are required at least in millimolar concentrations to induce bundling of FtsZ protofilaments in vitro (18, 19, 23). In this study, we show that ruthenium red, [(NH₄)₅Ru-O-Ru(NH₄)₅-O-Ru(NH₄)₅]Cl₆·4H₂O, can induce bundling of protofilaments at low micromolar concentrations. Ruthenium red is a water-soluble hexavalent polyoxometalate with several major uses in biological studies. In several proteins, ruthenium red competes for the calcium-binding site, and it is used as a probe for calcium (29, 30). It inhibits the actin-activated myosin Mg²⁺-ATPase in smooth muscle, perturbs the Ca²⁺ pump of the smooth muscle plasma membrane, and blocks the Ca²⁺-induced Ca²⁺ release from sarcoplasmic reticulum in both skeletal and cardiac muscle (31, 32). However, ruthenium red binds to tubulin at a site distinct from the high affinity calcium-binding site, and it is the only known ligand that binds to the α-tubulin subunit (33). Equimolecular concentrations of calcium and ruthenium red have been shown to inhibit microtubule polymerization completely and to disassemble the preformed microtubules (34). Here, we found that stoichiometric concentrations of ruthenium red induced the bundling of FtsZ protofilaments in the presence of GTP, enhanced sedimentable polymers mass, prevented polymer disassembly, and reduced the GTPase activity of FtsZ. Further, ruthenium red and calcium were found to share their binding sites on FtsZ.

EXPERIMENTAL PROCEDURES
Materials—Ruthenium red, guanosine 5′-triphosphate (GTP), 1,4-piperazinediethanesulfonic acid (PIPES), isopropyl-β-D-thiogalactopyranoside (IPTG), and bovine serum albumin.

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1 The abbreviations used are: GTP, guanosine 5′-triphosphate; PIPES, piperazine-1,4-bis(2-ethanesulfonic acid); BSA, bovine serum albumin.

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Purification of FtsZ—Recombinant Escherichia coli FtsZ was over-expressed and purified from E. coli BL21 strain (DE3) by using ion-exchange chromatography (diethylaminoethyl cellulose DE-52) followed by a temperature-dependent one-cycle glutamate-induced polymerization and depolymerization method, as described recently (19, 35). FtsZ concentration was measured by the Bradford method (41) using BSA as a standard and adjusting the final concentration of FtsZ using a correction factor of 0.82 for the FtsZ/BSA ratio (15). The purified protein was frozen and stored at −80 °C.

Preparation of Ruthenium Red—Ruthenium red was dissolved in 25 mM PIPES buffer, pH 6.8, and centrifuged at 52,000 × g for 30 min. The supernatant was passed through a 0.2 μm Millipore filter and stored at −80 °C.

Measurement of FtsZ Polymer Mass by Sedimentation Assay—FtsZ (7.3 μM) was polymerized in buffer A containing 1 mM GTP at 37 °C for 15 min at 37 °C. The polymers were sedimented at 30,000 × g for 20 min at 0 °C. The supernatants were incubated with 900 μM of ruthenium red in 52,000 × g at 0 °C for 15 min. The supernatants were incubated with 900 μM of ruthenium red in the presence of 10 mM calcium chloride for 30 min. The incorporation stoichiometry was calculated by subtracting the supernatant FtsZ concentration from the total FtsZ concentration. Further, the stoichiometry of incorporation of ruthenium red in the presence of 10 mM calcium chloride was determined.

Stability of FtsZ Polymers in the Presence of Calcium and Ruthenium Red—FtsZ (30 μM) was polymerized in the presence of 5 mM magnesium chloride, 10 mM calcium chloride, and 1 mM GTP for 15 min at 37 °C. The polymeric suspension of FtsZ was then dialyzed for 30 min against PIPES buffer containing different concentrations of calcium (0–10 mM) and ruthenium red (0–10 μM). The diluted polymeric suspensions were incubated for an additional 10 min at 37 °C. The polymers were sedimented at 52,000 × g for 20 min. The fraction of total FtsZ precipitated in the presence of different concentrations of calcium and ruthenium red was determined by Bradford reagent, as described in the previous paragraph.

Visualization of FtsZ Polymers by Electron Microscopy—Samples were prepared for electron microscopy as described in our recent report with minor modification (19) and inspected by using a Philips CS 200 electron microscope. Briefly, FtsZ (7.3 μM) in buffer A was polymerized with 1 mM GTP in the absence of calcium and magnesium and centrifuged at 52,000 × g for 10 min. The polymerized suspensions were negatively stained with 1% uranyl acetate and blotted dry. All of the electron micrographs were taken at 50,000× magnification.

GTPase Activity Measurement of FtsZ—The production of inorganic phosphate during GTP hydrolysis was measured by a standard malachite green solution method (19, 35). FtsZ (7.3 μM) in buffer A was incubated with GTP at different concentrations of calcium (0–10 mM) and magnesium (0–10 mM) at 37 °C for 10 min. Then, GTP (1 mM) was added to each sample, and the hydrolysis reaction was started by transferring the reaction mixtures to 37 °C. The GTP hydrolysis reaction was quenched at the desired time intervals by adding 10% (v/v) of 7% perchloric acid, and the quenched reaction solutions were kept at 0 °C until all of the time points were collected. The quenched reaction mixtures were then kept at 25 °C for 10 min and centrifuged for 10 min to remove aggregated proteins. 15 μl of supernatants were incubated with 900 μl of filtered malachite green sodium molybdate solution at 25 °C for 30 min. The production of phosphate ions was determined by measuring the absorbance at 650 nm. A phosphate standard curve was prepared using sodium phosphate.

RESULTS

Effects of Ruthenium Red on FtsZ Polymerization—FtsZ (7.3 μM) was incubated with 10 μM ruthenium red in 25 mM PIPES buffer for 30 min on ice. FtsZ-ruthenium red complex was then separated from the free ruthenium red molecules by passing the solution through a Sephadex G-25 column (12 × 1 cm). The fraction containing the highest FtsZ-ruthenium red was dialyzed extensively against PIPES buffer at 4 °C. The concentration of bound ruthenium red was calculated using a molar absorption coefficient of 68,000 M⁻¹ cm⁻¹ at 535 nm. The incorporation stoichiometry was calculated by dividing the bound ruthenium red concentration by FtsZ concentration. Further, the stoichiometry of incorporation of ruthenium red in the presence of 10 mM calcium chloride was determined.

Determination of the Stoichiometry of Copolymerization of Ruthenium Red in FtsZ Polymers—FtsZ (7.3 μM) was polymerized with different concentrations (2–10 μM) of ruthenium red in the presence of 1 mM GTP and 2 mM magnesium at 37 °C for 15 min. FtsZ polymers were spun down (52,000 × g at 30 °C) to separate free ruthenium red molecules from the polymer-bound ruthenium red. The polymeric FtsZ was dissolved in PIPES buffer. The concentration of polymer-bound ruthenium red was determined after gel filtration. After gel filtration, FtsZ-ruthenium red complex was dialyzed extensively for 24 h at 4 °C against PIPES buffer in the presence and absence of 10 mM calcium chloride. The incorporation stoichiometry of ruthenium red per FtsZ was determined as described earlier.

Visualization of FtsZ Polymers by Electron Microscopy—FtsZ (7.3 μM) was polymerized in the presence of different concentrations of calcium (0–10 mM) and magnesium (0–10 mM) and 1 μM GTP for 15 min. The polymers were negatively stained with 1% uranyl acetate and blotted dry. All of the electron micrographs were taken at 50,000× magnification.
significantly increased the efficiency of pelleting FtsZ polymers. For example, 2 μM ruthenium red increased the fraction of total pelleted FtsZ by 50% compared with control (in the absence of ruthenium red). At a relatively higher ratio of FtsZ:ruthenium red (1:1.4), the amount of pelleted polymer was increased by a factor of 2.

Electron microscopic studies showed an increase in FtsZ protofilaments bundling with an increasing concentration of ruthenium red (Fig. 3). Only a few tiny polymers of FtsZ were observed in the absence of ruthenium red (Fig. 3A), whereas a network of protofilament bundles was detected in the presence of 2 μM ruthenium red (Fig. 3B). More discrete and long protofilaments were visible with 4 μM ruthenium red, indicating a further increase in the protofilament bundling (data not shown). 10 μM of ruthenium red produced a dense network of thick polymers of FtsZ (Fig. 3C). Interestingly, the amount of polymer sedimented in the presence of 4 and 10 μM of ruthenium red were similar; however, 10 μM ruthenium red produced a significantly larger light-scattering signal than that produced by 4 μM ruthenium red. Thus, the increased light-scattering signal was due to extensive bundling of FtsZ polymers in the presence of 10 μM ruthenium red (Fig. 3C).
FIG. 4. Characterization of ruthenium red-induced FtsZ polymerization. FtsZ (7.3 μM) was polymerized in PIPES buffer containing 10 μM ruthenium red in the absence of GTP (○), in the absence of magnesium (Δ), and in the presence of 1 mM GTP and 2 mM magnesium (▲). The light-scattering signals were corrected by subtracting appropriate blank signals.

relatively high concentration (25 μM), ruthenium red induced extensive aggregation of FtsZ, but filamentous structures were not visible under the conditions used (data not shown). The results of light scattering and electron microscopic studies together demonstrated that FtsZ assembly occurred with a primary assembly of protofilaments followed by ruthenium red-induced bundling.

Is Ruthenium Red-induced FtsZ Polymerization GTP-dependent?—FtsZ (7.3 μM) was polymerized in buffer A containing 10 μM ruthenium red in the absence and presence of 1 mM GTP at 37 °C. There was no significant enhancement of the light scattering in the absence of GTP (Fig. 4). However, in the presence of GTP, light scattering increased to the same extent as that described in Fig. 1, suggesting that GTP was essential for ruthenium red-induced FtsZ polymerization (Fig. 4).

Ruthenium Red-induced FtsZ Polymerization Requires Magnesium—Because ruthenium red-induced assembly of FtsZ was performed in PIPES buffer containing 2 mM magnesium, we enquired whether magnesium was a requirement for the modulation of FtsZ assembly by ruthenium red. FtsZ was incubated with 10 μM ruthenium red in the absence and presence of 2 mM magnesium on ice. Polymerization reaction was started by adding 1 mM GTP to the reaction mixture and immediately transferring the solution to a cuvette at 37 °C. Ruthenium red produced minimal light-scattering signal in the absence of divalent magnesium, whereas it produced a large light-scattering signal in the presence of 2 mM magnesium (Fig. 4). Further, in the absence of magnesium, only 11% of the total FtsZ was pelleted in the presence of 10 μM ruthenium red. In addition, mostly aggregates of FtsZ and a very few short and thin protofilaments of FtsZ but no bundles were observed in the absence of divalent magnesium by electron microscopy at 50,000× magnification (data not shown). Furthermore, incubation of FtsZ with 10 μM ruthenium red in the absence of both divalent magnesium and GTP produced only aggregates, indicating that GTP hydrolysis was required for the filamentous assembly (data not shown). The GTPase activity of FtsZ was measured in the presence of 10 μM ruthenium red and 1 mM GTP in the absence of divalent magnesium at 37 °C. Ruthenium red did not induce the GTPase activity of FtsZ in the absence of magnesium, suggesting that magnesium was required for promoting the GTPase activity of FtsZ (data not shown). The results together suggested that ruthenium red required divalent magnesium for promoting filamentous assembly of FtsZ and subsequent bundling of FtsZ filaments.

Effect of Ruthenium Red on the GTPase Activity of FtsZ—Ruthenium red reduced the GTPase activity of FtsZ in a concentration-dependent manner (Fig. 5). 2 μM ruthenium red reduced the GTPase activity of FtsZ by 55%, compared with control (in the absence of ruthenium red), and 75% inhibition of the GTPase activity occurred in the presence of 10 μM ruthenium red. Ruthenium red was also found to induce bundling of FtsZ protofilaments, which could be due to the reduction of GTPase activity (Fig. 3). Alternatively, the bundling of protofilaments by ruthenium red could reduce the GTPase activity of FtsZ.

Does Ruthenium Red Copolymerize with FtsZ?—FtsZ (7.3 μM) was polymerized with different concentrations (2–10 μM) of ruthenium red, and the polymer-bound ruthenium red molecules were separated from the free ruthenium red molecules by pelleting down the FtsZ polymers. The polymeric pellets of FtsZ were dissolved in PIPES buffer, and the concentrations of ruthenium red and FtsZ were determined as described under “Experimental Procedures.” The stoichiometries of incorporation of ruthenium red per FtsZ molecule in the polymer were found to be 0.36 ± 0.01, 0.63 ± 0.13, and 0.82 ± 0.06 in the presence of 2, 6, and 10 μM ruthenium red, respectively (Table I).

Comparison of the Effects of Divalent Calcium and Ruthenium Red on the Assembly Properties of FtsZ—We examined the effects of calcium on the polymerization reaction of FtsZ exactly under the same conditions as described previously for ruthenium red and compared it with the effects of ruthenium red on FtsZ polymerization. Similar to previously reported findings (18), our results also showed that millimolar concentrations of divalent calcium enhanced the sedimentable polymer mass in a concentration-dependent manner (Fig. 6). For example, 1 and 5 mM calcium increased the sedimentable polymer mass by 30 and 52%, respectively, compared with the control (Fig. 6). The polymerization reaction reached the optimum at 10 mM calcium. However, ruthenium red exerted sim-
ilar effects on FtsZ polymer mass at micromolar concentrations (Fig. 2). For instance, 10 mM calcium increased the pelletable polymer mass by 85%, whereas only 10 μM ruthenium red was sufficient to increase the pelletable polymer mass by 98% (Figs. 2 and 6). Thus, divalent calcium required a 1000-fold higher concentration than ruthenium red to produce similar increase in the polymer level.

Further, we compared the relative potencies of calcium and ruthenium red to prevent dilution-induced disassembly of the preformed FtsZ polymers by a sedimentation assay. FtsZ (30 μM) was polymerized in the presence of 10 mM calcium and 1 mM GTP for 15 min at 37 °C. The polymer mass was quantified as described under “Experimental Procedures.”

![Graph](https://example.com/graph.png)

**Fig. 6. Calcium-induced polymerization of FtsZ.** FtsZ (7.3 μM) in buffer A was polymerized in the presence of different concentrations (0–10 mM) of calcium and 1 mM GTP for 15 min at 37 °C. The polymer mass was quantified as described under “Experimental Procedures.”

The data indicated that the bundling of protofilaments is favored at higher FtsZ concentrations. The preformed FtsZ polymers were diluted 30-fold into warm 25 mM PIPES buffer containing different concentrations of ruthenium red or divalent calcium. After an additional 10 min of incubation either with ruthenium red or divalent calcium at 37 °C, the polymers were sedimented. In the absence of the cations, only 8 ± 3% of the total FtsZ was pelleted as polymers. However, ~60% of the total protein was pelleted when 7.3 μM FtsZ was used for polymerization (Fig. 6). The results taken together showed that the fraction of total FtsZ pelleted increased at a higher concentration of FtsZ, which is consistent with a previous report (13). The data indicated that the bundling of protofilaments is favored at higher FtsZ concentrations. The preformed FtsZ polymers were diluted 30-fold into warm 25 mM PIPES buffer containing different concentrations of ruthenium red or divalent calcium. After an additional 10 min of incubation either with ruthenium red or divalent calcium at 37 °C, the polymers were sedimented. In the absence of the cations, only 8 ± 3% of the total FtsZ was pelleted as polymers, indicating that the FtsZ polymers were highly labile. The sedimented protein concentrations in the pellet fractions were found to increase with increasing concentration of ruthenium red (Fig. 7A). For example, 18 ± 5%, 50 ± 6%, and 70 ± 3% of the total FtsZ were pelleted in the presence of 1, 2, and 10 μM of ruthenium red, respectively. Thus, ruthenium red strongly stabilized FtsZ polymers against dilution-induced disassembly. Like ruthenium red, divalent calcium also increased the pelletable polymer mass in a concentration-dependent manner (Fig. 7B). For example, 19 ± 5% of total FtsZ was pelleted in the presence of 5 mM calcium, and 52 ± 2% FtsZ was pelleted in the presence of 10 mM calcium. Thus, similar to ruthenium red, calcium also stabilized FtsZ polymers against dilution-induced disassembly; however, calcium required a 1000-fold higher concentration than ruthenium red to produce similar stabilizing effects upon FtsZ polymers.

**Does Ruthenium Red Bind at the Calcium Binding Site(s) of FtsZ?**—We used two approaches to find out whether ruthenium red shares its binding site on FtsZ with calcium. In the first approach, FtsZ was polymerized in the presence of ruthenium red with or without 10 mM calcium. After an additional 10 min of incubation either with ruthenium red or divalent calcium at 37 °C, the polymers were sedimented. In the absence of the cations, only 8 ± 3% of the total FtsZ was pelleted as polymers, indicating that the FtsZ polymers were highly labile. The sedimented protein concentrations in the pellet fractions were found to increase with increasing concentration of ruthenium red (Fig. 7A). For example, 18 ± 5%, 50 ± 6%, and 70 ± 3% of the total FtsZ were pelleted in the presence of 1, 2, and 10 μM of ruthenium red, respectively. Thus, ruthenium red strongly stabilized FtsZ polymers against dilution-induced disassembly. Like ruthenium red, divalent calcium also increased the pelletable polymer mass in a concentration-dependent manner (Fig. 7B). For example, 19 ± 5% of total FtsZ was pelleted in the presence of 5 mM calcium, and 52 ± 2% FtsZ was pelleted in the presence of 10 mM calcium. Thus, similar to ruthenium red, calcium also stabilized FtsZ polymers against dilution-induced disassembly; however, calcium required a 1000-fold higher concentration than ruthenium red to produce similar stabilizing effects upon FtsZ polymers.

**FIG. 6.** Calcium-induced polymerization of FtsZ. FtsZ (7.3 μM) in buffer A was polymerized in the presence of different concentrations (0–10 mM) of calcium and 1 mM GTP for 15 min at 37 °C. The polymer mass was quantified as described under “Experimental Procedures.”

**FIG. 7.** Stabilization of FtsZ polymers against dilution-induced disassembly. **A** and **B**, the fraction of total FtsZ pelleted in the presence of different concentrations of ruthenium red and calcium, respectively. The fraction of total protein pelleted was determined as described in Fig. 2. The data represent an average of four individual experiments.

**TABLE I**

| Ruthenium red | Calcium | Incorporation stoichiometry |
|---------------|---------|------------------------------|
| μM | mM |  |
| 2 | 0 | 0.36 ± 0.01a |
| 4 | 0 | 0.55 ± 0.12 |
| 6 | 0 | 0.63 ± 0.13 |
| 10 | 0 | 0.82 ± 0.06 |
| 10 | 10 | 0.20 ± 0.03 |

a ±, standard deviations.
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I). In the second approach, FtsZ was incubated with 10 μM ruthenium red without GTP for 30 min in the absence and presence of 10 mM divalent calcium at 0°C. The stoichiometry of ruthenium red incorporated per soluble FtsZ was determined after gel filtration and exhaustive dialysis. The stoichiometries of incorporation of ruthenium red per monomer of FtsZ were found to be 0.7 and 0.3 in the absence and presence of 10 mM calcium, respectively. Calcium also displaced ruthenium red from the preformed FtsZ-ruthenium red complex. For example, the stoichiometry of ruthenium red incorporation per FtsZ was reduced by 55% by 10 mM calcium. The results together suggested that ruthenium red and calcium share their binding sites on FtsZ, and that ruthenium red could be used as a probe to determine the binding site of calcium on FtsZ and the role of calcium on FtsZ assembly (29, 30).

DISCUSSION

The intensity of light scattering depends upon the polymer mass as well as the size and shape of the polymers (36). Under the conditions used in this study, FtsZ formed small single protofilaments in the absence of ruthenium red, and these thin polymers did not scatter light efficiently. Ruthenium red increased the light-scattering signal of FtsZ assembly in a biphasic manner, suggesting that the assembly of FtsZ occurred in two steps in the presence of ruthenium red. The first step involved the assembly of FtsZ into protofilaments, and then ruthenium red induced the bundling of single protofilaments that scattered light intensely. The bundling of protofilaments increased the size of the polymers that not only assisted the visualisation of polymers by electron microscopy, but also facilitated efficient pelleting of protofilaments, which were otherwise difficult to pellet. For example, even using 280,000 x g, only 27% of the total FtsZ was sedimented in the presence of 2.5 mM magnesium, whereas 50 and 51% of the total FtsZ were sedimented in the presence of 10 mM magnesium (12, 22). Alternatively, small single protofilaments of FtsZ that were formed in the absence of ruthenium red or calcium disassembled during the centrifugation. The cations prevented depolymerization of the protofilaments by stabilizing the polymers, which, in turn, increased the pelleted polymeric mass. Therefore, although it seemed that ruthenium red and calcium increased the polymeric mass of FtsZ, the actual polymeric mass, i.e. assembled protofilaments, might be nearly the same with or without the cations. However, we could not rule out the possibility that the observed increase in the polymeric mass in the presence of ruthenium red was at least partly due to the promotion of FtsZ assembly.

The polycation DEAE-dextran and lipid monolayer have been shown to assemble FtsZ into large protofilaments, sheets, and minirings in the absence of GTP (11). In contrast to the stimulating effects of DEAE-dextran on both FtsZ and microtubule polymerization, ruthenium red promoted FtsZ assembly, but it inhibited microtubule polymerization (34, 37). DEAE-dextran has multiple positive charges, and it is an extremely large molecule (molecular weight = 500,000), ~12 times bigger in size than FtsZ. Thus, it is reasonable to consider that the binding sites of DEAE-dextran and ruthenium red on FtsZ are also different. Further, ruthenium red needed GTP and magnesium to induce FtsZ assembly, whereas DEAE-dextran promotes assembly in the absence of GTP, suggesting that DEAE-dextran and ruthenium red induce FtsZ assembly by different mechanisms.

Ruthenium red completely inhibits microtubule assembly at stoichiometric concentrations and binds to tubulin tightly (33, 34). Ward et al. (38) suggested that ruthenium red binds to the negatively charged carboxyl-terminal domain of the α-tubulin. Ruthenium red did not bind to the calcium-binding site on tubulin, suggesting that they have different binding sites on tubulin (33, 34). However, calcium and ruthenium red disassembled microtubules and inhibited microtubule polymerization (33, 34). Surprisingly, both calcium and ruthenium red promoted FtsZ assembly in a similar fashion. The differential behavior of calcium and ruthenium red toward the polymerization of tubulin and FtsZ might be due to the different binding interactions of these metal ions with tubulin and FtsZ. Like tubulin, FtsZ also has a negatively charged carboxyl-terminal tail. Ma and Margolin (39) found that the C-terminal-truncated FtsZ polymerized in the presence of calcium and suggested that calcium has a binding site on FtsZ which is not located at the truncated C-terminal. Thus, the binding site of calcium on FtsZ appears to be different from its binding site on tubulin. We found that calcium inhibited the binding of ruthenium red to FtsZ, suggesting that ruthenium red shares its binding site on FtsZ with calcium. Therefore, it is possible that, similar to calcium, ruthenium red also binds to a site on FtsZ that is not located at the C terminus.

Substoichiometric incorporation of ruthenium red into FtsZ polymers strongly reduced the GTP hydrolysis rate and stabilized the polymers against disassembly (Table I; Figs. 5 and 7). The bundling of FtsZ polymers could be due to an increase in the lateral interaction among the protofilaments in the presence of ruthenium red. Increased lateral interaction among the protofilaments reduced the solvent-accessible surface area around the nucleotide-binding site on FtsZ that was thought to inhibit the rate of subunit exchange (40). Thus, the reduced GTPase activity of FtsZ could be due to a strong stabilization of FtsZ polymers in the presence of ruthenium red. Alternatively, the reduced GTPase activity could increase the stability of the polymers in the presence of ruthenium red.

The strong effects of ruthenium red on FtsZ assembly indicated the presence of an important regulatory site on FtsZ. It has been hypothesized that carefully balanced polymer stability plays an important role in the positional regulation of FtsZ. It is conceivable that, similar to the fine regulation of actin and microtubule polymerization by their accessory proteins, FtsZ assembly is also finely regulated by its associated proteins. A few proteins have already been identified that regulate FtsZ assembly and its functional organization (1, 2, 24–26). For example, it has been shown that a basic protein ZipA promotes and stabilizes protofilament assembly of FtsZ (25, 26). Further, ZipA assists in regulating FtsZ protofilaments into arrays of long bundles or sheets and plays a critical role in the organization of the FtsZ ring in bacterial cells (25, 26). Thus, it is tempting to speculate that ruthenium red may mimic the action of a positively charged peptide or a protein that regulates the assembly dynamics of FtsZ in bacteria.

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