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Single tryptophan mutants of FtsZ: Nucleotide binding/exchange and conformational transitions

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ABSTRACT

Cell division protein FtsZ cooperatively self-assembles into straight filaments when bound to GTP. A set of conformational changes that are linked to FtsZ GTPase activity are involved in the transition from straight to curved filaments that eventually disassemble. In this work, we characterized the fluorescence of single Trp mutants as a reporter of the predicted conformational changes between the GDP- and GTP-states of *Escherichia coli* FtsZ. Steady-state fluorescence characterization showed the Trp senses different environments and displays low solvent accessibility. Time-resolved fluorescence data indicated that the main conformational changes in FtsZ occur at the interaction surface between the N and C domains, but also minor rearrangements were detected in the bulk of the N domain. Surprisingly, despite its location near the bottom protofilament interface at the C domain, the Trp 275 fluorescence lifetime did not report changes between the GDP and GTP states. The equilibrium unfolding of FtsZ features an intermediate that is stabilized by the nucleotide bound in the N-domain as well as by quaternary protein–protein interactions. In this context, we characterized the unfolding of the Trp mutants using time-resolved fluorescence and phasor plot analysis. A novel picture of the structural transition from the native state in the absence of denaturant, to the solvent-exposed unfolded state is presented. Taken together our results show that conformational changes between the GDP and GTP states of FtsZ, such as those observed in FtsZ unfolding, are restricted to the interaction surface between the N and C domains.

1. Introduction

Cell division protein FtsZ polymerizes at the middle of the cell to form the Z-ring which provides constriction force for initiation of cytokinesis [1]. In vitro, FtsZ bound to GTP self-assembles into straight filaments and magnesium-induced hydrolysis of the nucleotide drives depolymerization [2,3]. Electron microscopy shows that stabilized FtsZ-GDP polymers adopt a helix-like shape that differs from the straight polymers observed in solutions of FtsZ-GTP [4,5]. These findings suggest a different conformation of the monomer depending on the bound nucleotide, acting as switch in the assembly-disassembly process that drives Z-ring constriction. The crystal structure of FtsZ shows two globular domains, the nucleotide binding N domain and the catalytic C domain, that are connected by the central H7 helix [6]. Although many crystals of FtsZ have been reported to date, all were considered to be in the curved form [7,8]. Only recently was a clear change in FtsZ conformation, regarded as the straight form, reported in the crystal structure of *Staphylococcus aureus* FtsZ in complex with the cell-division inhibitor PC190723, [9,10]. Computational analyzes on FtsZ crystals described flexible segments in the FtsZ sequence mainly located in the C domain [11–13]. Martín-Galiano et al. [12] identified FtsZ mutations located between H7 helix and the C domain that presumably blocked the switch from straight to curved conformations. We investigated the disrupting effect of Trp mutations on the FtsZ structure and function (*Escherichia coli* FtsZ lacks Trp residues in its sequence) and found that tyrosine in position 222 is a key residue in FtsZ polymerization (unpublished data and [11]). More recently, Chen and Erickson [14] characterized the same mutation, Y222W, and based on fluorescence quenching experiments, proposed a movement leading to a separation of the N and C domains that followed FtsZ assembly. These accumulated data indicated that the nucleotide-mediated conformational changes

Abbreviations: GDP, GTP, Guanosine diphosphate, triphosphate; Trp, Tryptophan; GdmCl, Guanidinium chloride

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attributed to the transition from straight to curved filaments involve hinge-like movements between N and C domains. In fact, the structure of the FtsZ-PC190723 complex shows a striking new orientation between the otherwise structurally unmodified N and C domains, in accordance with experiments.

In this work, we used time-resolved fluorescence of FtsZ Trp mutants as a reporter of the local conformational transitions related to the nucleotide bound state at equilibrium. A FtsZ structural model is depicted in Fig. 1, where the nucleotide binding domain (N domain) is colored blue, the catalytic domain (C domain) is colored red, and the plane of interaction between both domains is indicated by the H7-helix, colored in cyan. The Trp mutants were selected based on their locations in the FtsZ structure. As seen in the model, the F40W mutation (side chain in orange) is located on the nucleotide binding domain; the Y222W mutation (side chain in green) is located on the interface between both domains, and the F275W mutation (side chain in yellow) is located on the catalytic domain. This study was extended to characterization of the conformational transitions in FtsZ unfolding, which we previously described using the classic a linear extrapolation method [11,15,16]. In the past, we have applied time-resolved fluorescence methods to study protein conformational changes and unfolding [17–19], and more recently, we exploited the phasor method to describe complex fluorescence decays of fluorophore mixtures and of biological samples [20,21]. Here, we describe the application of the phasor plot method to the time-resolved fluorescence of FtsZ Trp mutants.

2. Materials and methods

2.1. Protein purification and additional methods

Details on protein purification, determination of protein concentration and nucleotide content, measurement of secondary structure by CD and measurement of mant-GDP and mant-GTP dissociation constants can be found in the supporting information.

2.2. GTPase activity and polymerization

The GTPase activity and polymerization of FtsZ wt and of the Trp mutants were measured by the malachite green colorimetric assay and by 90° angle light scattering at 350 nm, respectively, as previously described [22]. The reaction mixtures contained FtsZ wt or the Trp mutants at desired protein concentration dissolved in 50 mM Mes-KOH pH 6.5, 50 mM KCl, and 10 mM $MgCl_2$ and were incubated at 30 °C. The polymerization reaction was started by the addition of 0.5 mM GTP.

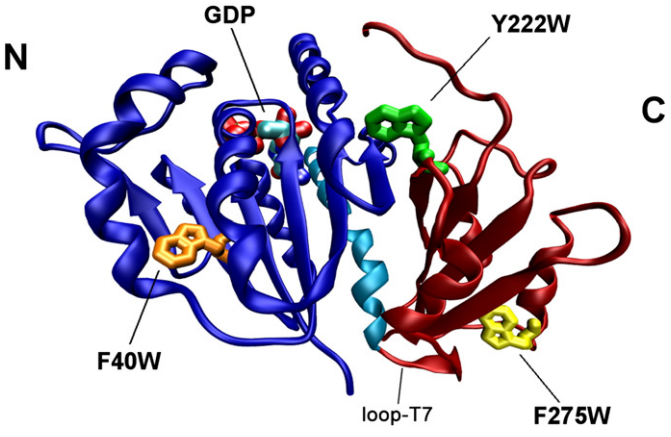

Fig. 1. FtsZ structure showing Trp introduced by mutagenesis. FtsZ structural model showing the position of the three tryptophans introduced by site-directed mutagenesis. The nucleotide binding domain (N) and the catalytic domain (C) were colored in blue and red, respectively. The H7 helix is considered the boundary between both domains and was colored in cyan. For simplicity, the three tryptophans introduced at positions 40 (orange), 222 (green) and 275 (yellow) are all shown in the same molecule. This figure was prepared using V.M.D. [36] and refined with POV-Ray version 3.6 (Persistence Of Vision Raytracer Pty, Ltd.).

2.3. Tryptophan steady-state fluorescence

Corrected emission spectra of total protein fluorescence were measured on an ISS PC1 spectrofluorimeter (ISS, Champaign, IL) using 275 nm (16 nm bandpass) as the excitation wavelength. For quenching experiments, emission spectra were recorded on a Perkin-Elmer LS-50 spectrofluorimeter (Perkin-Elmer, Waltham, MA) excited at 295 nm. Spectroscopic grade acrylamide (Merck, Germany) was added to protein mixtures in aliquots from a 1 M stock solution. Fluorescence intensities were corrected for dilution effects. The proteins stocks were dialyzed against 50 mM potassium phosphate buffer pH 6.5 before adjusting the concentration to the indicated values. The temperature was kept constant at 25 °C using a circulating water bath. All measurements were performed in 10 × 4 mm quartz cuvettes.

2.4. Tryptophan time-resolved fluorescence

Frequency domain time-resolved fluorescence was recorded using an ISS Chronos fluorometer. Samples were excited using a 300-nm LED in conjunction with a 295-nm band pass interference filter (Semrock, Rochester, NY), resulting in a nominal excitation maximum of 298-nm. Using a magic-angle polarizer configuration (vertical excitation polarization and 55° emission polarizer), the emission was collected through two 320-nm long pass filters to block scattered light. Proteins stocks were dialyzed against 50 mM potassium phosphate buffer pH 6.5. Then, the absorbance at the nominal excitation wavelength was adjusted to be just below 0.1 to avoid inner filter effects, resulting in protein concentration ~50 μM. N-acetyl-L-tryptophanamide (Sigma, St. Louis, MO) dissolved in pH 6.5 buffer was used as the reference lifetime, with an assigned 3.0 ns monoexponential decay. In the unfolding experiments, denaturant concentration was adjusted by adding aliquots from 6 M stock solutions. Measurements were performed in 10 × 4 mm quartz cuvettes at 25 °C. Fluorescence lifetimes were fit using GLOBALS for spectroscopy software (http:/www.lfd.uci.edu/globals/).

2.5. Phasor plots

The phasor method for representing time-resolved fluorescence has been reviewed elsewhere [21,23]. However, a brief description of the method is given here. The phasor approach offers a model-less method to visually assess aspects of excited state lifetimes. In particular, if the excited state decay is mono-exponential, the phasor point, for any light modulation frequency, will lie on the so-called “universal circle”, while the phasor points for multi-exponential decays will lie inside this circle (see Results). Complex decays may thus be displayed by a single point in phasor-space, which may be monitored to follow the effects of perturbations, such as structural transitions or the addition of denaturants, on the excited state properties. Experimental data used to build the phasor plots were obtained under the same conditions described above for ‘Tryptophan time-resolved fluorescence’. For further details on construction of phasor plots, see supporting information.

3. Results

3.1. In vitro activity and secondary structure of FtsZ wt and Trp mutants

We compared the homogeneity and oligomerization state of FtsZ wt and Trp mutant protein preparations using Coomassie blue-stained SDS-PAGE and analytical size-exclusion chromatography, respectively (Figure S1). For all proteins, densitometry analysis of the gel revealed a minimum 94% signal corresponding to the FtsZ band. Some differences were observed in the oligomerization state, varying within 92–97% monomer populations. Under these experimental conditions in the