Characterization of F2C-FtsZ on DOPC/PE-MCC supported lipid bilayer

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Abstract

We present the characterization of F2C-FtsZ (referred as Z2) on DOPC/PE-MCC supported lipid bilayer system. The characterization was performed using Atomic Force Microscopy (AFM) and Quartz Crystal Microbalance (QCM). AFM images were performed in the absence and presence of guanosine 5'-triphosphate (GTP). In the absence of GTP, Z2 protein on lipid bilayer images are visually indistinguishable from the lipidic bilayer images, but the image analysis shows that Z2 protein on lipid bilayer samples present a lower roughness than lipidic bilayer. QCM was unable to detect the adsorption of protein into the lipidic bilayer. AFM images in the presence of 10 mM GTP show a filament growth rate of 3.3 nm·min$^{-1}$ in average, the FtsZ filaments grow in two directions, apparently led by the atomic structure of the mica. The characterized system can be used for studying FtsZ filaments polymorphism, filament polymerization rates and polymerization led by solid substrates.

1 Introduction

The bacterial cytoskeletal protein FtsZ, prokaryotic homologue of tubulin, plays an essential role in cytokinesis. FtsZ forms a ring (the Z ring) under the membrane at the center of the cell, and this Z ring constricts to initiate division of the cell.[1] FtsZ has GTPase activity, and is composed by two globular domains, N-terminal domain and C-terminal domain, these two domains are separated by a central core helix H7. [2] The N-terminal has the nucleotide binding site and the C-terminal domain has the catalytic loop T7. When FtsZ polimerize the synergic loop, T7, inserts into the nucleotide site of the following subunit placing the catalytic residue near the $\gamma$-phosphate allowing thus nucleotide hydrolysis.[3] In vitro experiments show that in presence of guanosine 5'-triphosphate (GTP), FtsZ can condense into a large variety of higher-order structures depending on the medium conditions.[1] Not only the medium conditions are important to describe the morphology diversity of FtsZ polymers, FtsZ and lipids have a tendency to interact amongst themselves. In artificially membrane-bound FtsZ, depending on the tether of FtsZ to the membrane of liposomes, Z-rings produce convex protrusions if the tether is switched to the N-terminus, or concave depressions if the tether is switched to the C-terminus. [4] Additionally, the combination of elements like filament curvature, twist and the strength and orientation of its surface attachment can explain the morphology diversity of FtsZ filaments, and how these elements can modulate the force exerted on the membrane during cell division. [5] In order to understand these elements and their interactions, we have characterized the reconstituted system that is composed by a solid support, a lipid bilayer with two lipids, one of them has a maleimide group, and the mutant F2C-FtsZ protein (referred as Z2) with a cysteine positioned in the second aa in the N-terminal domain and the mutatnt F2C-FtsZ protein (referred as Z2) with a cysteine positioned in the second aa in the N-terminal domain that allow the binding to the maleimide-modified lipid bilayer via maleimide-cysteine reaction that forms a stable thioether linkage that is not reversible. [6] The characterization of the system was performed with AFM technique to follow in real time the protein polymerization with nanometric resolution.

2 Methods

- Atomic force microscopy (AFM)

AFM techniques are normally used for surface imaging. Length and height measurements for surface features can be easily performed on micrometric and nanometric scale. [7] AFM allows scanning samples in air, vacuum, different gases, or liquids, becoming an important technique to study biological systems under physiological conditions. [8]
AFM is an important technique to follow dynamic biological processes taking place in real time, and conformational changes of proteins and their interactions with other proteins.[9]

Atomic force microscopy was performed on the lipid bilayer-anchored Z2 in buffer Z in absence of GTP at room temperature, in order to trigger the polymerization 10 mM of GTP was added to the buffer Z and the temperature was decreased until 15°C. AFM images were recorded with a microscope from Agilent 5500 AFM operated in tapping mode in a liquid environment cell with temperature control. Image analysis was performed with WSxM software. [10] The sample for AFM (Fig. 1) can be summarized as follows.

![Figure 1: a) DOPC. b) PE-MCC](image)

**Figure 1:** a) DOPC. b) PE-MCC

Liposome preparation. Two separate stock solutions of the lipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC, 786.113 Da) (Fig. 1a) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidomethyl)cyclohexane-carboxamide] (PE-MCC, 985.252 Da) (Fig. 1b), both of Avanti Polar Lipids Inc., were solubilized in a mixture of chloroform:methanol (1:1, v/v) and stored at -20°C. In order to obtain large unilamellar vesicles (LUVs) of mixture of DOPC:PE-MCC (9:1, mol/mol), we used the extrusion method: i) the lipid mixture was evaporated under nitrogen stream into a round bottomed flask by 30 minutes until a thin layer of lipids forms in the bottom, ii) the lipids were resuspended and vortexed by 30 minutes in 250 µL of buffer solution A [50 mM Tris-Cl (pH 6.8)] at a final lipid concentration of 2 mg·mL$^{-1}$, iii) finally the the suspension was extruded 31 times through a 200 nm pore membrane.

**Preparation of Lipid Bilayer.** To fuse the lipid bilayer on the solid substrate, the liposomes solution was diluted in buffer B [50 mM Tris-HCl (pH 6.8), 200 mM NaCl, 2 mM CaCl$_2$] at 0.1 mg·mL$^{-1}$ and placed over cleaved mica for 45 minutes at 30°C, then the sample was rinsed with buffer Z [50 mM Tris-HCl (pH 7), 500 mM KCl, 5 mM MgCl$_2$] to remove excess LUVs.

**Incubation of protein FtsZ2.** Protein solution at 5 mM were incubated on the formed bilayer for 2 hours to ensure complete coverage of the active surface. In order to prevent disulfide bonds between proteins and the consequent protein self-aggregation, 100 µM of TCEP was added.

![Figure 2: Sketch of sample for AFM](image)

**Figure 2:** Sketch of sample for AFM

- **Quartz crystal microbalance (QCM)**

QCM devices allow the study of viscoelastic properties of matter, the adsorption of molecules, or the motility of living cells.[11] QCM analysis utilizes the resonance frequency shifts of a thickness shear mode resonator oscillating at 5 MHz in response to the deposition of a foreign mass, such as a thin organic layer.[12] The frequency decrease is essentially proportional to an increase in film thickness in the case of a thin rigid monolayer.[13]

Before begin the experiment, it is necessary to prepare the substrate. We used a SiO$_2$ substrate that was cleaned for 15 minutes in SDS 2% (w/v),
then washed with distilled water Milli-Q and dried under a stream of nitrogen. After cleaning, the substrate was activated by exposing it to UV for 1 hour in order to eliminate organic contaminants and oxidize the silicon surface rendering it hydrophilic. The QCM experiment can be summarized as follows:

1. **Acquisition of a baseline.** The chamber was loaded with buffer A which was kept at a constant flow for 15 minutes to ensure a baseline.

2. **Liposome injection.** A liposome solution diluted in buffer B at 0.1 mg·mL⁻¹ final concentration was injected for about 15 minutes until a lipid bilayer was obtained, then the chamber was rinsed with the same buffer B for 10 minutes to get the change in frequency only due to the mass adsorbed to the sensor.

3. **Protein injection.** The chamber was loaded with buffer Z which was kept at a constant flow for 15 minutes, then the protein was injected at two concentrations: 2 µM for 15 minutes and, 5 µM for 100 minutes, then the chamber was rinsed with buffer Z for 15 minutes.

### 3 Results and discussion

**Direction of filaments growth led by atomic structure of mica.** The crystal structure of muscovite mica consists of complicated aluminosilicate layers separated by K⁺ ions, the mica surface is composed of of Si (partially Al) and O atoms forming an array of hexagons with a unit cell length of 0.52 nm. [14] The direction of filaments growth follows two directions, when each hexagon of mica is orientated 90° (Fig. 3b) blue hexagon) the filaments grow at 27.7 ± 5.1° and 89.1 ± 4.1° following two sides of the mica hexagon with 30° and 90°, respectively, and when each hexagon of mica is orientated 180° (Fig. 3d) blue hexagon) the filaments grow at 61.9 ± 4.0° and 130.5 ± 5.3° following two sides of the mica hexagon with 60° and 120°, respectively. Although the interaction between the atomic structure of mica and the protein, through the lipidic bilayer is unknown, the atomic structure of the substrate seems modulate the direction of protein polymerization.

**Filament growth rate.** Two AFM images (Fig. 4) with an interval of 96 minutes between them were used to determine the growth rate of 131 filaments, two directions were used (∼30° and ∼90°). The growth rate of the ∼30° was 3.29 ± 1.82 nm·min⁻¹ and 3.31 ± 1.77 nm·min⁻¹ for ∼90° filaments. Tukey’s test analysis between the two directions shows that the growth rate differences were not significant. The filament growth rate is surprisingly low compared to 6-subunit-thick bundles growth rate of ∼20 µm·s⁻¹·µM⁻¹. [15] These six orders of magnitude difference could be due to restrictions imposed by the linker (PE-MCC) to the protein.

**QCM measures.** The formation of lipid bi-
layer of DOPC/PE-MCC on SiO2 substrate (Fig. 5) indicates a resonance frequency change of -28.6 ± 0.3 Hz, which corresponds to a mass increase per unit area of 494 ± 4 ng·cm⁻², and a dissipation value < 0.75 × 10⁻⁶. The adsorption of Z2 to the lipid bilayer of DOPC/PE-MCC (Fig. 6) indicates a resonance frequency change of -1.11 ± 0.23 Hz, which corresponds to a mass increase per unit area of 0.49 ± 0.10 pmol·cm⁻², giving a diameter per molecule on the surface of ~21 nm. This diameter does not correspond with the FtsZ monomer diameter of ~4.5 nm.[1]. For a ~4.5 nm monomer diameter we expected a resonance frequency change of ~23 Hz, value that corresponds a FtsZ monolayer adsorbed.

Figure 5: Formation of lipid bilayer of DOPC/PE-MCC on SiO2 substrate.

Figure 6: Adsorption of Z2 to the lipid bilayer of DOPC/PE-MC.

In addition to the inability of the QCM for detection of protein binding to the bilayer, the AFM images of a specific sample do not show the structure of the FtsZ protein (Fig. 7a,b) on the lipid bilayer in absence of GTP. Profiles of AFM images (Fig. 7c,d) show differences in surface roughness depending of system composing, lipid bilayer on mica has RMS roughness value of ±0.1536 nm and lipid bilayer with Z2 has a RMS roughness value of ±0.0741 nm. Only when we add GTP and decrease the temperature at 15° the polymerization begins and we can see the protein structure on the lipid bilayer. (Fig. 8). The apparent lack of protein on the bilayer may be due to the lipid of the linker, PE, with an inverted truncated cone structure [16] that could forms depressions on the bilayer where the protein is located.

Figure 7: a)Lipid bilayer on mica. b) Lipid bilayer with Z2 protein. c) Profile of line A (image a). d) Profile of line B (image b)

Figure 8: a)10×10 µm AFM image of Z2 on DOPC/PE-MCC. b) 500×500 nm AFM image of Z2 on DOPC/PE-MCC.

4 Conclusions

FtsZ attached to lipidic bilayer was achieved using the mutatnt F2C-FtsZ protein with a cysteine positioned in the second aa in the N-terminal domain, and a lipid bilayer composed of two lipid, DOPC and PE-MCC. The PE-MCC lipid has a
maleimide group that specifically binds to the cysteine of the protein. Given that the lipids occupy an area of $\sim 0.5 \text{ nm}^2$ and the protein occupies an area of $\sim 20 \text{ nm}^2$,[17] 40 lipid molecules needed to cover the area occupied by a molecule of protein. The molar ratio 9:1 (DOPC/PE-MCC) ensures that for each protein reaches the bilayer surface, four PE-MCC lipids are available to anchor the protein. Although the QCM is unable to detect protein adsorption to the lipid bilayer and AFM images of samples in the absence of GTP do not show the structure of the protein on the bilayer, we know that the protein is present because adding GTP and decrease the temperature, FtsZ polymerizes forming straight filaments. The characteristics of the system, such as low filaments growth rate and the likely directed polymerization offers several advantages for the study of polymorphism present in FtsZ filaments. There is a particular interest in using the system described here to demonstrate the existence of torsion between monomers in FtsZ filaments, this could be achieved by adapting the system to curved surfaces on which the filaments present its preferred curvature and torsion angle. Another possible application of the system to demonstrate the torsion is the use filaments Z2 anchored to the bilayer as binding sites for free FtsZwt (without cysteine). FtsZwt does not have the restriction imposed by the anchor to the bilayer, thus it is expected that the straight filaments described in the system became in curve filaments by interleaving the free protein.

References


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