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Computer aided design of FtsZ targeting oligopeptides[†]

Stefano Pieraccini^{*a,b}, Stefano Rendine^{#a}, Chacko Jobichen^{#c}, Prerna Domadia^c, J. Sivaraman^c, Pierangelo Francescato^a, Giovanna Speranza^{a,b}, and Maurizio Sironi^{*a,b}

^aDipartimento di Chimica and INSTM - UdR, Università degli Studi di Milano, Via Golgi 19, 20133, Milano, Italy

^bIstituto di Scienze e tecnologie molecolari del CNR (CNR-ISTM), Via Golgi 19, 20133, Milano, Italy. stefano.pieraccini@unimi.it; maurizio.sironi@unimi.it

^cDepartment of Biological Science, National University of Singapore, 14 Science Drive 4, 117543, Singapore

[#] These authors contributed equally to this work.

Abstract

FtsZ is a protein involved in the bacterial division process and is thus an emerging target for antibacterial drugs. The network of interactions between FtsZ monomers necessary for exploitation of its biological function are studied here with molecular dynamics and free energy calculations. The results obtained led to the design of FtsZ targeting peptides which exhibited activity against the function of FtsZ *in vitro*.

The development of new strains of bacteria resistant to most antibiotics is causing growing alarm in the medical community.¹ Nonetheless, only few novel antibacterial drugs have been recently marketed or are in an advanced clinical trial phase. The FtsZ protein has raised increasing interest as an innovative antibacterial drug target since it is a highly conserved protein present in most prokaryotes. FtsZ is a distant homolog of the eukaryotic protein tubulin,² and has similar functions, as it is involved in the cell duplication process. In particular FtsZ self assembles to form the Z-ring,³ a complex structure which allows bacterial septation and then cell division. Interfering with FtsZ self assembly is thus an eligible way to stop bacterial proliferation.

Numerous FtsZ targeting small molecules have been described so far,^{4–7} mainly derived from natural sources and identified by high throughput screening. A few peptidic inhibitors have also been studied, such as Edeine B1,⁸ the synthetic peptide MCiZ⁹ and Stathmine derived peptides.¹⁰

A viable approach toward the alteration of FtsZ polymerisation and Z-ring formation is to interfere with the protein-protein interactions responsible for Z-ring self assembly. The design of molecules targeting protein-protein interactions (PPIs), albeit proven to be a very complex task due to the peculiar features of protein interfaces and their inherent variability,

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¹¹ has lately gained increasing interest as a therapeutic strategy.^{12,13} It has been pointed out that in general only a minor part of the residues making up the interface actually contribute to the protein-protein binding energy,¹⁴ thus molecules designed to target either the critical areas where those residues are located or their binding sites would be likely to interfere with the protein assembly process.¹⁵ Moreover, it has been argued that molecules designed to target PPIs would be particularly useful in the development of antibiotics and antiviral agents, due to the low similarity between microbial and human protein-protein interfaces, and to the high conservation of protein contact surfaces.¹⁶

Here, we describe an *in silico* study of the network of interactions between two bound FtsZ monomers, performed through computational alanine scanning (CAS).¹⁷ The aim of the CAS is to map the residues at the protein interface that contribute to the binding energy, which are called “hot spots”. These residues are critical for protein-protein interactions and form structural motifs which can be considered as the binding epitopes. If a potential binding epitope is made up of residues that are next to one another not only in the topology but also in the sequence of the protein, then peptides can be synthesised to match the identified motif. Such peptides could therefore competitively inhibit FtsZ polymerisation by selectively binding to protein sites critical for the self assembly process. With respect to most molecules affecting FtsZ function, that target the nucleotide binding site or the large cleft between the nucleotide binding site and the GTPase activating domain,⁷ we aim at designing peptides binding at the contact surface between FtsZ molecules, thus modulating the polymerisation process *via* a direct inhibition of the PPI between FtsZ monomers.

The model system we simulated is a *Mycobacterium tuberculosis* FtsZ homodimer composed of two monomeric subunits with identical sequences. They are here labelled A and B (orange and green respectively) in Fig. 1a to distinguish them. The initial structure of FtsZ was obtained from the Protein Data Bank (PDB ID 1RLU¹⁸). A molecular dynamics simulation of the FtsZ complex was run in explicit solvent with periodic boundary conditions for 5 ns. CAS analyses were performed using the MM-GBSA technique¹⁹ on 300 snapshots extracted from the last 3 ns of the simulation, after equilibration was reached. Full details of the simulation protocols are given in the supplementary materials.[†] The CAS results show that the hot spots are evenly distributed on the A and B subunits and arranged in a complementary fashion. In particular three hot spots were identified, conveniently next to one another both in the tertiary and in the primary structure of the protein, namely Met₁₇₇, Phe₁₈₀ and Arg₁₈₁, belonging to the H1 helix of the B subunit and located at the interface between the two FtsZ monomers. We thus identified an octapeptide, (**1**) see Chart 1, corresponding to the FtsZ subsequence ranging from Ser₁₇₅ to Ser₁₈₂ and containing the three hot spots, as a possible candidate to be an inhibitor of FtsZ polymerization. To verify this hypothesis a molecular dynamics simulation of (**1**) bound to the FtsZ subunit A was run. The helical structure of (**1**) was conserved and the CAS results showed that its hot spots were preserved during the simulation. The binding free energy change associated to Arg₁₈₁ mutation into alanine is indeed increased in (**1**) with respect to the dimer (Fig. S1, ESI[†]). In Fig. 1c the formation of a stable salt bridge between Arg₁₈₁ and Asp₉₄′ (residues on the A subunit are denoted with a prime) is shown. The binding free energy upon mutation of Arg₁₈₁ is consequently hugely incremented compared to the dimer (Fig. 1b), where this salt

bridge was observed only for a small fraction of the simulation. In the dimeric complex Arg₁₈₁ oscillates between Asp₉₄ and Asp₁₇₈, the latter being located on the same subunit as the arginine residue, therefore not contributing to the binding energy between the two FtsZ subunits.

Small peptides are prone to losing their secondary structure when they are extracted from their protein environment, and also (**1**), albeit retaining its helical structure when in complex with FtsZ, was structureless when simulated in water solvent (Fig. S2, ESI[†]). It is therefore expedient to constrain its conformation so as to make the helical structure stable.

Interestingly, during the MD simulation of the complex (**1**)-FtsZ, a hydrogen bond (HB) between the sidechains of Asp₁₇₈ and Ser₁₈₂ was detected (Fig. S3, ESI[†]). These residues do not interact with the A FtsZ subunit neither in the dimer nor in the protein-peptide complex. The aforementioned HB leads to the formation of a virtual 19 membered ring involving all the amino acids between Asp₁₇₈ and Ser₁₈₂ and gives a hint on how to constrain the helical structure with simple chemical modifications. We mutated *in silico* Ser₁₈₂ into an ornithine and cross-linked its sidechain with that of Asp₁₇₈ with an amide bond, thus obtaining a covalently bonded cyclic structure with the same topology of the H-bonded one (Fig. 1d). The resulting cyclic peptide, (**1a**), was then simulated both free and in complex with FtsZ. The helical conformation of the free peptide was highly stabilised by the proposed modification, while the (**1a**)-FtsZ complex showed no modification of the key interactions with the protein compared to the linear analogue (Fig. 1d). Another serine, *i.e.* Ser₁₇₅ is present in (**1**). Albeit only occasionally H-bonding with Asp₁₇₈ during our simulations, a cyclic peptide, named (**1b**), was designed with the previously described strategy. Peptide (**1b**) also displayed enhanced secondary structure stability and no modification of its interactions with FtsZ with respect to (**1**) (Fig. S1, S2, S4, ESI[†]).

In order to assess the potential inhibitory effects of the designed peptides, they were synthesised and their ability to affect FtsZ polymerisation was tested *in vitro*. Since FtsZ polymerization is strictly GTP dependent and its assembly/disassembly process depends on the rate of GTPase hydrolysis, monitoring the amount of inorganic phosphate (P_i) released upon treatment of *E.coli* FtsZ with the selected peptides provides a direct measure of the rate and extent of inhibition of FtsZ polymerization exerted by the peptides. In control FtsZ, the number of P_i ions released due to rapid GTP hydrolysis increased up to 10 min, roughly paralleling the rapid polymerization. Thereafter a steady-state phase up to 60 min was attained as the hydrolysis rate plateaus due to GTP consumption. We also tested an inactive octapeptide, not representing the hot spot, for GTPase assay. As seen in Fig. 2, at 20 min of hydrolysis, this inactive octapeptide showed no inhibition of the GTPase activity as compared to the other three peptides which remarkably inhibited the extent of GTP hydrolysis of FtsZ by 100%. Even at other time intervals, *i.e.* 10 min, 40 min and 60 min, GTPase inhibition induced by the inactive octapeptide was not significant, *i.e.* 16%, 9% and 29%, respectively. In the control without any inhibitory peptides, FtsZ polymers were formed in abundance even after 20 min. On the other hand, upon treatment of FtsZ with the peptides, strong perturbation of FtsZ GTPase activity by maximal six to seven fold was observed (Fig. 2). Linear peptide (**1**) completely inhibits GTP hydrolysis of FtsZ at 20 min as well as by 63%, 80% and 93% at 10 min, 40 min and 60 min, respectively. Cyclic peptide

(1a) abolishes the initial rate of GTP hydrolysis by ~100% from the values ($\pm$ s.e.m.) of $7.65 \pm 1.5 \text{ min}^{-1}$ and $4.82 \pm 0.55 \text{ min}^{-1}$ in the absence of peptide to $0.11 \pm 0.28 \text{ min}^{-1}$ and $0.02 \pm 0.32 \text{ min}^{-1}$ at 10 min and 20 min, respectively. Whereas (1b) strikingly suppresses the rate of GTP hydrolysis by 100% in the steady-state phase from the values ($\pm$ s.e.m.) of $6.52 \pm 1.3 \text{ min}^{-1}$ and $6.3 \pm 1.34 \text{ min}^{-1}$ in the absence of peptide to $-0.11 \pm 0.72 \text{ min}^{-1}$ and $-0.08 \pm 0.59 \text{ min}^{-1}$ at 40 min and 60 min, respectively. This study clearly shows that the inhibition of GTPase activity by linear and cyclic peptides strongly affects the *E.coli* FtsZ dynamics.

To further explore the effect of the peptides on the FtsZ assembly and to identify the related changes in polymer morphology, transmission electron microscopy (TEM) imaging of *E.coli* FtsZ was performed as described in the supplementary material. FtsZ protein was incubated with or without peptides and visualized for TEM imaging (Fig. 3). Control FtsZ readily assembles into an abundant, thick and dense polymeric network of protofilaments which are mostly straight, although a mild degree of curvature is observed (Fig. 3a). A 5 μM concentration of each of the three peptides could readily prevent the formation of the thick and dense filamentous crowding noted for control FtsZ. Peptide (1), after 5 min showed very few, isolated, thin and curved FtsZ polymers (Fig. 3b). In the presence of cyclic peptide (1b), polymer morphology was also clearly perturbed and few short, thin and highly curved FtsZ polymers, analogous to typical GDP-liganded FtsZ, were formed (Fig. 3c). Cyclic peptide (1a) prevented the formation of FtsZ higher order assemblies such as bundles or extensive networks of protofilaments (Fig. 3d).

Both the cyclic peptides, at 5 and 10 μM respectively, were also able to destabilize and disrupt typical 'preformed' GTP-FtsZ protofilaments under similar conditions and induced the formation of ill-shaped protofilaments like those described above (data not shown). Full details of the experimental procedures are found in supplementary materials.[†] An inactive octapeptide control, not representing the hot spot, was also included in this study. It showed no effect on the inhibition of FtsZ protofilaments, promoting formation of filamentous crowding (Fig. 3e). The effect of a well known inhibitor of FtsZ polymerization, the small molecule berberine,²¹ is shown in Fig. 3f for comparison.

In conclusion, we identified the hot spot on the proteinprotein interface between FtsZ subunits and used the derived interactions map to select an octapeptide, corresponding to an FtsZ subsequence, which could interfere with FtsZ self assembly. This peptide was then modified through two different schemes of cyclisation to constrain its helical geometry. Cyclic peptides have high GTPase activity and are notably successful in inhibiting the rapid initial polymer formation and causing depolymerization in response to the reduction in GTP in the initial steady phase of polymerization. Since these peptides interfere with both the assembly and the enzyme activity of *E. Coli* FtsZ, they can be the starting point for the future development of a new class of antimicrobials targeting cell division, acting by blocking the FtsZ function *via* peptidomimetism. Further studies involving structure-guided peptide and peptidomimetic synthesis will be able to optimize the properties of these compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Global structure of the *Mycobacterium tuberculosis* homodimer (a). The two subunits, labelled A and B in the text, are depicted in orange and green respectively. The region containing the most significant hot spots is drawn in red. A zoomed in image of the hot region (b). Significant salt bridges (green dashed lines) and hydrophobic interactions (blue dashed lines) are outlined. Peptide (1) in complex with FtsZ (c). Cyclic peptide (1a) in complex with FtsZ (d). Figures are rendered with VMD.²⁰

Fig. 2.

Time course of GTPase activity with or without the addition of peptides. The effect of the peptides on the relative GTPase activity of FtsZ (6 μ M) assembled in MES buffer was determined using the malachite green/sodium molybdate assay. The mean of duplicate experiments, repeated twice, was plotted against time. Error bars represent the standard error of the mean. (P < 0.02 ***, P < 0.05 **, P < 0.2 * in comparison with the control.)

Fig. 3.

Visualization of FtsZ assembly with or without addition of peptides by TEM. Electron micrographs of negatively stained FtsZ polymers were observed under a transmission electron microscope at $\times 20000$ magnification, shown as absence (A) and presence of $5\ \mu\text{M}$ linear peptide (1) (B) $5\ \mu\text{M}$ cyclic peptide (1b) (C) $5\ \mu\text{M}$ cyclic peptide (1a) (D) $5\ \mu\text{M}$ inactive octapeptide control (E) and $100\ \mu\text{M}$ berberine (positive inhibitor) (F). Typical polymer morphology change involved is pinpointed by arrows. Scale bar, $1\ \mu\text{m}$ (A-C), $500\ \text{nm}$ (D-E) and $100\ \text{nm}$ (F).

Ac-Ser-Leu-Met-Asp-Ala-Phe-Arg-Ser-NH₂ (1)

Ac-Ser-Leu-Met-Asp-Ala-Phe-Arg-Orn-NH₂ (1a)

Ac-Orn-Leu-Met-Asp-Ala-Phe-Arg-Ser-NH₂ (1b)

Chart 1