FtsZ Bacterial Cytoskeletal Polymers on Curved Surfaces: The Importance of Lateral Interactions

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ABSTRACT A recent theoretical article provided a mechanical explanation for the formation of cytoskeletal rings and helices in bacteria assuming that these shapes arise, at least in part, from the interaction of the inherent mechanical properties of the protein polymers and the constraints imposed by the curved cell membrane (Andrews, S., and A. P. Arkin. 2007. *Biophys. J.* 93:1872–1884). Due to the lack of experimental data regarding the bending rigidity and preferential bond angles of bacterial polymers, the authors explored their model over wide ranges of preferred curvature values. In this letter, we present the shape diagram of the FtsZ bacterial polymer on a curved surface but now including recent experimental data on the in vitro formed FtsZ polymers. The lateral interactions between filaments observed experimentally change qualitatively the shape diagram and indicate that the formation of rings over spirals is more energetically favored than estimated in the above-mentioned article.

Received for publication 22 December 2007 and in final form 7 March 2008.

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In recent years, it has become evident that the bacterial cell contains a large number of organized elements that fit within the wide concept of cytoskeleton used in eukaryotes: stable or dynamic polymers of filamentous nature with long-range order that can assemble, disassemble, and redistribute rapidly within the cell in response to signals that regulate cellular functions (1,2). Some of these polymers are bound to the inside of the cell membrane, forming rings or helices (3–6), and they can serve different functions that go from helping define the cell morphology (7); locating the cell division site (2); to forming a ring that contracts during cell division (8,9). A recent theoretical analysis (10) characterizes the shape of the membrane-bound polymers with a sequence of turning angles α_ϕ, α_θ, and α_ψ (see Fig. 1 *D*). The energy was taken as a sum of independent contributions for each bond along the filament, assuming quadratic dependence of the bond energy with the deviations of the three angles with respect to their optimal values α_ϕ^o, α_θ^o, and α_ψ^o. Then, the minimum energy structures were morphologically associated to the cytoskeletal rings and helices formed by different bacterial proteins and used to estimate the preferential bond angles between these proteins.

Here we focus our attention on one of the first bacterial cytoskeletal proteins that has been described: the polymers formed by FtsZ, the bacterial ancestor of the eukaryotic tubulin and essential component of the division machinery (9,11). We incorporate our previously obtained experimental data, indicating the importance of the lateral interactions between filaments, into the theoretical analysis describing the shapes adopted by the membrane-bound polymers. We observe that the shape-diagram is significantly modified and the ring conformation is more energetically favorable than described in Andrews and Arkin (10). The optimization of the conformation of the preferential polymeric bond angles presented in Andrews and Arkin (10) yields that only a very restrictive condition, $\alpha_\psi^o = 0$ and $\alpha_\theta^o < 0$, would allow a ring conformation of the FtsZ filament on the cylindrical bacteria. Any positive (or negative) value of α_ψ^o would produce a right- or left-hand helix, as the optimal configuration (Fig. 4, *C* and *D*, and Table I in (10); and our Fig. 1 *A*). However, an analysis including the lateral interactions observed in vitro provides a much wider range of angles compatible with the formation of the ring structures observed in vivo.

Atomic force microscopy allowed the first observation of the dynamic behavior of the polymers adsorbed on a surface immersed in solution (12). Individual *Escherichia coli* FtsZ filaments were seen to be highly flexible, capable of fragmenting and reannealing, curving in the presence of GTP and GTP analogs, and showed a strong tendency to establish lateral contacts. Theoretical analysis (13) permitted the first estimation of polymer properties extracted from experimental data: the preferential angle $\alpha_\phi^o = 2.5°$ between the monomers, the flexural rigidity $\kappa_\phi = 890\ kT$, and at the same time, the importance of the lateral attractions to stabilize the observed rolled configurations, with attractive energy per monomer estimated to be $\varepsilon = 2\ kT$ in a tightly bound roll. The picture that emerged is that of "linear aggregates" (which does not mean straight polymers but assemblies formed by sequential addition of monomers to the growing polymer) with a hierarchical structure in which the lateral attractions

Editor: Kathleen B. Hall.

doi: 10.1529/biophysj.107.128363

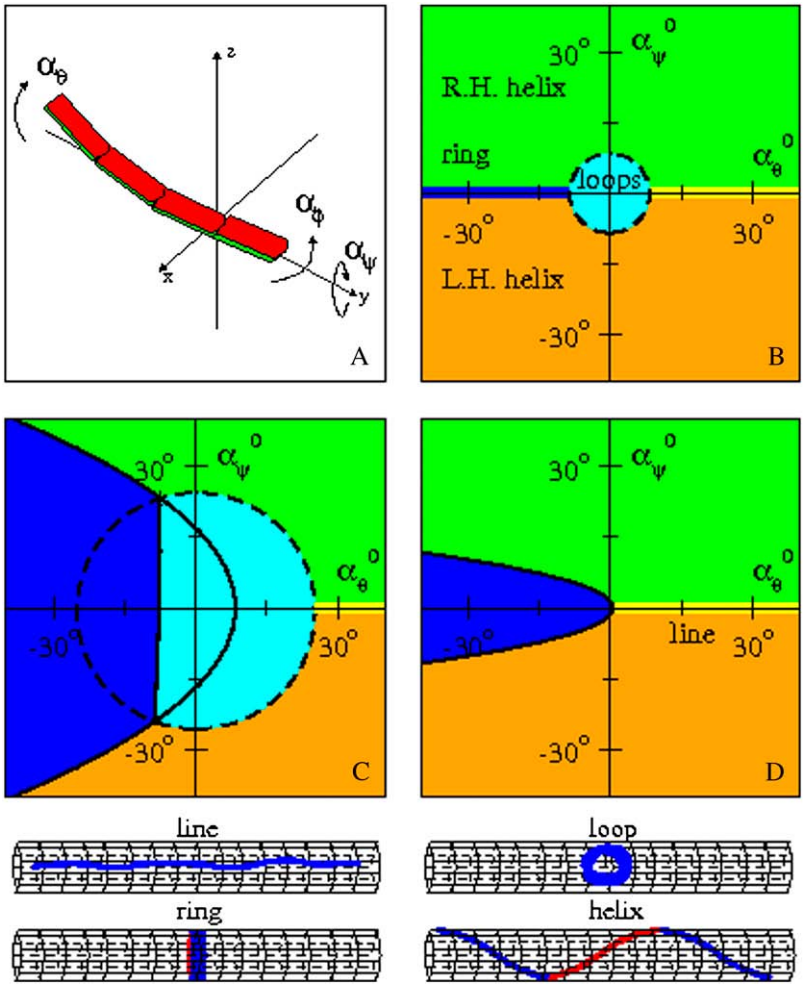

FIGURE 1 Shape diagrams for filaments with 3000 FtsZ monomers anchored to a cylindrical surface with radius *R*. The optimal configurations: line, helix, loop, and ring are sketched at the bottom, and they are found in different regions of the (α_θ°, α_ψ°) plane, for the optimal bond angles between the monomers, described in panel *A*. Panel *B* corresponds to the analysis in Andrews and Arkin (10), without any lateral interaction of the filaments. Panel *C* uses the experimental values for FtsZ filaments on mica, with an attractive interaction $\varepsilon = 2$ *kT* per monomer, and preferential angle $\alpha_\phi^\circ = 2.5^\circ$. Panel *D* describes the effect of filament bundling, with a severe reduction of the attraction effects to $\varepsilon = 0.2$ *kT*, and making $\alpha_\phi^\circ = 0$.

are important, although significantly weaker than the bond energy between monomers. The modulation of lateral interactions by experimental conditions (temperature, pH, etc.) or physiological ligands or proteins can promote, with a very small free energy perturbation, changes that bring about large changes in the geometry of the polymers, explaining the extensive polymorphism of FtsZ polymers observed (11,12,14–16).

The experimental evidence for lateral attraction in FtsZ filaments changes qualitatively the shape-diagram in Andrews and Arkin (10). The shape of a protein filament on a curved membrane would be a balance between the optimization of the bond angles along the filament, and the global effects of the lateral interactions between those parts of the filament which are brought together by a possible folding of the filament in rings or loops. Fig. 1 presents the equivalent shape diagram for a filament with $N = 3000$ subunits on a curved membrane, taking into account the optimized bond angles along the filament and the global effects of the lateral interactions between filaments. We use the experimental data for α_ϕ° and ε, together with the known size of the FtsZ monomers and the radius $R \approx 500$ nm of the *E. coli* cylindrical membrane. The number of subunits used is also of the same order of magnitude of the estimated 5000–15,000 FtsZ monomers estimated to be present in bacterial cells (11). The result is that the ring configuration becomes stable with respect to the open helix within a large parabolic region over the unknown parameters α_θ° and α_ψ°. Therefore, the value $\alpha_\psi^\circ = 0$ for FtsZ, as given in Table I in Andrews and Arkin (10), cannot be inferred from the experimental observation of such FtsZ-rings. A wide range of optimal bond angles would end in similar ring structures as the result of the lateral attractions.

Our experimental data come from the analysis of individual filaments. Several proteins that interact with FtsZ and excluded volume conditions (14,16) are known to induce filament bundling. This would reduce the effective strength of the lateral interactions and could suppress filament preferential curvature by braiding of individual filaments. Fig. 1 *C* explores the structures formed under the assumption that the lateral interactions are reduced to a 10th of the observed value on mica, and the curvature suppressed. The

later assumption leaves only helices and rings, which are the predominant structures observed in vivo (1,2,4). Even under these circumstances, the formation of rings is predominant over helix formation for a wide range of optimal bond angles.

The presence of lateral interactions between FtsZ filaments has strong implications. It accounts for the fact that ring structures are more energetically favorable than estimated by Andrews and Arkin (10), which is compatible with the evidence that rings are preferentially formed in vivo. Lateral interactions could also underlie a GTP-independent force generating mechanism (13). The suggested mechanism is compatible with recent structural evidence indicating that filaments can be wrapped around the cylinder as spirals and not as a closed ring (17), with experimental results that highlight the physiological relevance of lateral interactions (18), and the fact that GTP hydrolysis is apparently not essential for constriction (8).

ACKNOWLEDGMENTS

We thank Jesus Mingorance for useful comments.

This work was supported by grant Nos. FIS2004-05035-C03-02 (to P.T.), BFU2005-0487-C02-01 (to G.R.), and C02-02 (to M.V.) from the Ministerio de Educación y Ciencia; grant Nos. S-0505/ESP-0299 (P.T.), S-0505/MAT-0283 (to M.V.), and S-BIO_0260/60 (to G.R.) from the Comunidad Autónoma de Madrid; and grant No. BIOSINCEL CSIC PIF06-004 (to G.R.).

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