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Molecular dynamics simulation of GTPase activity in polymers of the cell division protein FtsZ

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ABSTRACT

FtsZ, the prokaryotic ortholog of tubulin, assembles into polymers in the bacterial division ring. The interfaces between monomers contain a GTP molecule, but the relationship between polymerization and GTPase activity is not unequivocally proven. A set of short FtsZ polymers were modelled and the formation of active GTPase structures was monitored using molecular dynamics. Only the interfaces nearest the polymer ends exhibited an adequate geometry for GTP hydrolysis. Simulated conversion of interfaces from close-to-end to internal position and vice versa resulted in their spontaneous rearrangement between active and inactive conformations. This predicted behavior of FtsZ polymer ends was supported by in vitro experiments.

1. Introduction

FtsZ is the core protein in the process of bacterial division [1,2], being a main target for inhibitor molecules with promising use as future antimicrobials [3,4]. FtsZ assembles in the mid-cell into a large structure called the division-ring, which is formed of, among other proteins, polymers of FtsZ [5]. The structure of the monomer–monomer contacts in the FtsZ dimer, including the GTP site in the interface, was predicted (using 3D computer models based on the monomer crystal structure [6]) to be equivalent to its eukaryotic homologous structure, the tubulin α/β dimer [7]. This hypothesis was later confirmed by crystallography [8]. Several hypotheses have been posited to explain the role of GTP hydrolysis in the functionality of FtsZ [9–12]. A previous work using molecular dynamics (MD) simulations led to an explanation of the role of K^+ in the active centre, as well as the prediction, experimentally corroborated, that GTPase activity is dissociated from polymer formation [13]. Recently, analyzing substrate kinetics of the GTPase activity of *Escherichia coli* FtsZ, it has been concluded that GTPase active sites in FtsZ are independent of each other [14]. In this work, using MD simulations of modelled short polymers of FtsZ, we have studied the active/inactive state of monomer–monomer interfaces. In silico results were then tested by measuring the FtsZ GTPase-specific activity in vitro.

2. Materials and methods

2.1. 3D modelling and molecular dynamics

Three-dimensional models of FtsZ polymers were constructed by successive structural alignment of the two FtsZ subunits of the previously published MD-equilibrated dimer [13]. MD simulations were performed using the PMEMD module of the AMBER10 package (http://ambermd.org/; [15]). Each modelled FtsZ polymer was surrounded by a rectilinear solvent box with a minimum distance of 10 Å from the edge of the box to the closest atom of the solute, and with periodic boundary conditions, using LEAP. Adaptation to the AMBER force field was performed by 10000 steps of energy minimization using a cut-off of 12 Å and a δt of 0.002 ps. During the initial heating phase (200 ps), the temperature was raised from 0 to 300 K, restraining the position of the Cα atoms with a force constant of 20 kcal mol^{-1}, reducing the force constant

Abbreviation: MD, molecular dynamics

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in a stepwise fashion in the subsequent phase. After equilibration, unrestrained MD was performed for 8 ns in each case, relocating the hydrogen atoms using the SHAKE algorithm. The coordinates were saved for analysis of atom positions every 20 ps. Continuous tracking of the position of water molecules in the neighbourhood of the interfaces was performed using *Ptraj–watershell*.

2.2. Measurement of GTPase activity

E. coli FtsZ was purified by the Ca^{2+}-induced precipitation method [16]. Prior to use, protein was dialyzed in reaction buffer (5 mM $MgCl_2$, 250 mM KCl, 50 mM Tris pH 7.5) and then incubated at different concentrations with 1 mM GTP at 22 °C. Reaction was stopped by dilution in 65 mM EDTA in the same buffer, and phosphate concentration was determined by a colorimetric assay using the green malachite reagent [17,18]. Activity values were calculated by measuring the slope of the linear part of the activity curves.

3. Results and discussion

3.1. GTPase interfaces in FtsZ polymers

To study the behavior of GTPase interfaces in FtsZ polymers, a set of three-dimensional models of short FtsZ polymers (trimer, tetramer and pentamer) was generated using the equilibrated structure through MD procedures of the FtsZ dimer described previously [13] as the initial template. We used this model as starting point because it locates the side chain of some critical residues (i.e. the side chain of Asp residues 235, 238 and 72) in a position suitable for catalysis, solving some of the structural ambiguities exhibited by the original FtsZ dimer structure [8,19]. A scheme of an active interface is shown in Fig. 1. In order to evaluate the behavior of each active GTP centre, all systems were subjected to 8 ns of unrestrained MD. Sufficient sampling was evaluated as indicated in Supplementary data.

Throughout the MD, the position of the water molecules that came within 3.8 Å of the GTP molecules at the interfaces was continuously tracked. These included the two water molecules in the coordination sphere of the K^+ as well as all the other water molecules that eventually reached the active centre during the MD. Fixed water molecules in the coordination sphere of Mg^{2+} were excluded. For each water molecule traced, two different measurements were recorded (Fig. 1, inset): first the distance "*d*" from the oxygen atom of the water molecule (O_{wat}) to the phosphorus atom of the gamma phosphate (P_γ) of GTP and also the angle "α" between the axis defined by the same O_{wat} and P_γ atoms, and the axis defined by P_γ and the oxygen 3 atom of the beta phosphate ($O3_\beta$) of GTP. Only water molecules located at a distance of 3.0–3.8 Å from P_γ and at an angle greater than 110° with respect to the P_γ–$O3_\beta$ axis, can hydrolyze the GTP molecule [20–22]. Those FtsZ polymer interfaces containing water molecules that met these two conditions in a stable way were considered as "active interfaces" in terms of GTPase activity.

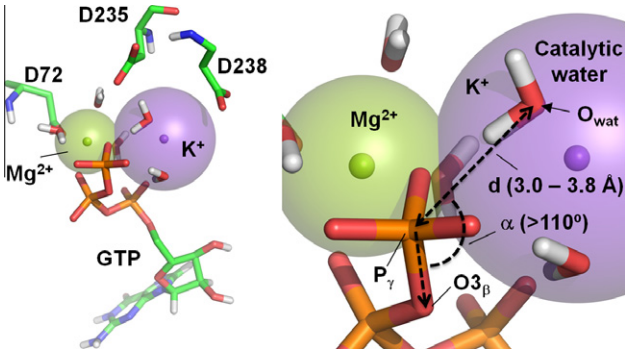

Fig. 1. Schematic representation of an active FtsZ dimer interface. GTP molecule (sticks) is associated to a Mg^{++} atom (green sphere) coordinating three molecules of water. Two additional water molecules are located in the coordination sphere of the K^+ atom (purple), including the catalytic water. Adequate "*d*" distance between O_{wat} and P_γ (3.0 Å < *d* < 3.8 Å) and minimum "α" angle among O_{wat}, P_γ and $O3_\beta$ (α > 110°) necessaries for a correct catalytic activity are indicated.

3.2. Trajectory analysis of GTPase interfaces during molecular dynamics of simulated FtsZ polymerization

Fig. 2A and Table 1 summarize the analysis of GTPase active and inactive interfaces in simulated FtsZ trimer, tetramer and pentamer (a complete continuous measurement of distance "*d*" and angle "α" of the water molecules at all GTP interfaces during MD

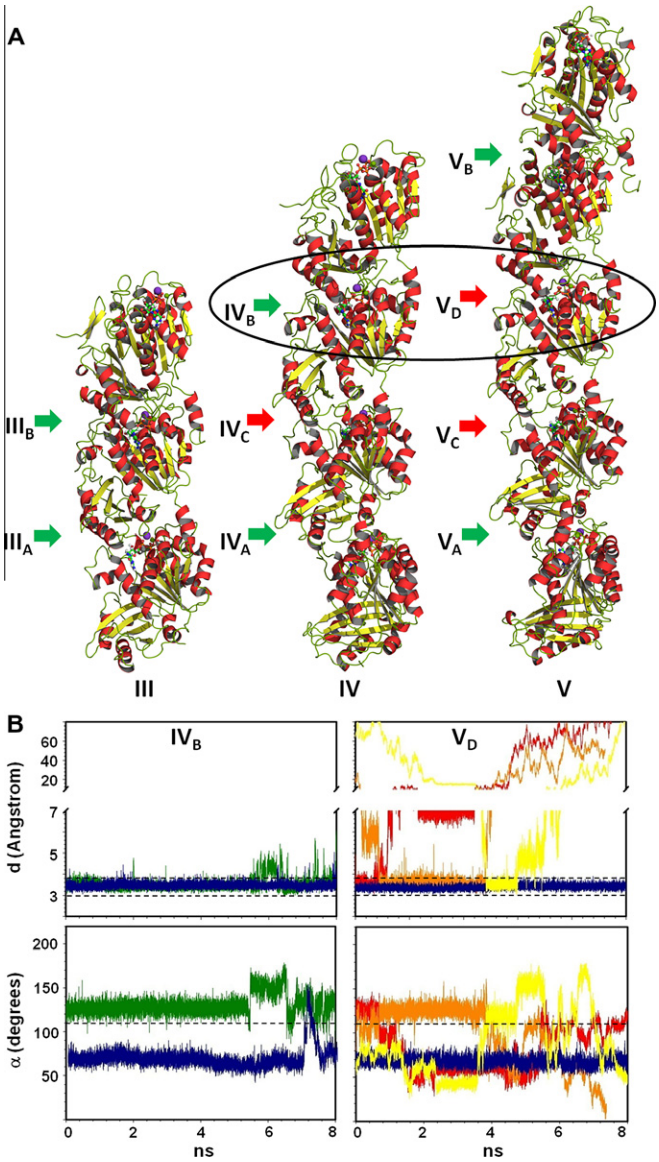

Fig. 2. (A) Representation of modelled FtsZ trimer (III), tetramer (IV) and pentamer (V). Arrows indicating the position of the interfaces are coloured according to their activity state (green: active; red: inactive). Transition from active to inactive state in the case of interfaces IV_B to V_D is highlighted. (B) Continuous measurement of "*d*" distance and "α" angle for all water molecules present in the interfaces IV_B and V_D over 8 ns of unrestricted MD. The range 3.0–3.8 Å, which corresponds to the optimal distance, and the position of minimum angle (110°) is indicated in each plot (dotted line).

Table 1
Maximum time (in percentage of total simulation time) of a water molecule located in a position compatible with GTP hydrolysis in each oligomer interface.

Trimer (III)	Tetramer (IV)	Pentamer (V)	Tetramer (IV′)
–	–	**V_B: 86%**	**IV′$_B$: 79%**
–	**IV_B: 88%**	V_D: 36%*	IV′$_D$: 16%
III_B: 92%	IV_C: 29%*	V_C: 09%	**IV′$_C$: 53%****
III_A: 86%	**IV_A: 92%**	**V_A: 98%**	–

In bold: interfaces predicted to be active.
* Active state was maintained only at the beginning of the MD, exhibiting gradual disordering along the trajectory (see Fig. 2 and Supplementary Figs. S1 and S2).
** Active state was stabilized during the second half of the trajectory, after gradual re-ordering (see Fig. 4 and Supplementary Figs. S1 and S2).

are shown in Supplementary Figs. S1 and S2). In the case of the FtsZ trimer (lane III), both interfaces contained water molecules positioned at a suitable distance and angle, which remained constant through almost the whole simulation, as previously described for the FtsZ dimer [13]. In the FtsZ tetramer, only the interfaces located near the ends (IV_A and IV_B), but not central interface (IV_C), had a water molecule positioned at the correct angle and distance for catalysis (i.e. dark green line in plot IV_B of Fig. 2B). In the pentamer, only interfaces V_A and V_B (nearest to the ends of the polymer) are active, whereas the central interfaces (V_C and V_D) do not maintain a statistically favourable active position for catalysis. In the interface V_D (equivalent to interface IV_B in the tetramer), five molecules of water circulate in the neighbourhood of P_γ in an unstable way (yellow to red lines in plot V_D of Fig. 2B), none of them remaining at the end of the trajectory in a stable position to lead to a catalytic event.

In summary, the simulations indicated that only those interfaces located next to the ends of the FtsZ polymers would be active, meaning that there are two active sites per filament.

A combination of factors could be the cause of the different behavior exhibited by the interfaces. The relative positions of all the residues located at the interfaces, their contact distances (including the ones used in [13]), as well as residues proposed to be related to monomer flexibility [11] were traced during the trajectory. None of them exhibited significant differences among interfaces during the MD procedure, so no individual parameter can be used as a marker for the active/inactive state of each. Alternatively, the putative movements associated with each protein segment in the polymers were modelled using normal mode analysis. The results suggested that the cause appeared to be more associated with the capacity of each monomer to adapt its movements to the adjacent monomers through the corresponding interface (Supplementary Fig. S3), maintaining the correct geometry of the active site.

3.3. GTPase specific activity of FtsZ in vitro

Consequently with the MD model, if the polymer length increases with the protein concentration, the specific GTPase activity of FtsZ should eventually decrease in parallel, because it is calculated dividing the activity by the total amount of protein, not by the number of active sites. To test this hypothesis, we measured the GTPase specific activity of FtsZ in vitro at different protein concentrations: from 1 to 40 μM. As shown in Fig. 3, once reached the critical concentration for polymerization (1–2 μM), there is an initial phase of rapid increase in GTPase specific activity at concentrations from 1 to 10 μM, which we interpret as resulting from the formation of FtsZ short polymers exhibiting the maximal specific activity (higher ratio of active vs. inactive interfaces). After this, and according to the model, a substantial decrease in specific activity was observed, lowering to 50% of the maximal value at a protein concentration of 40 μM. In previous work by Sossong et al. [10],

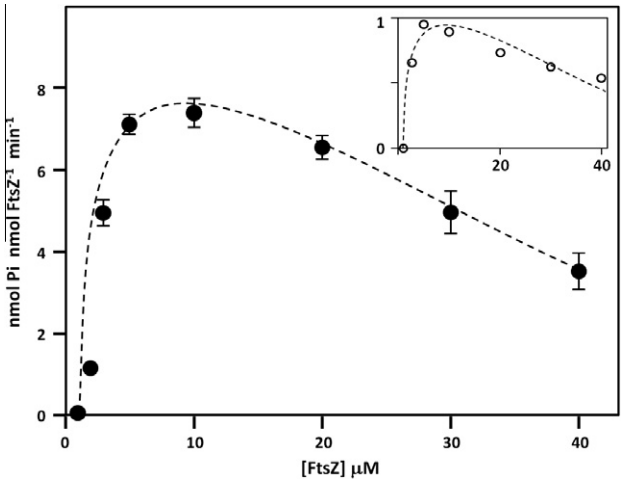

Fig. 3. Specific GTPase activity at increasing concentrations of FtsZ. Activity data (nmol Pi × nmol FtsZ^{-1} × min^{-1}) were calculated by measuring the slope of the linear part of the activity curves in the presence of GTP. Values are the mean ± standard deviation of four independent experiments. Inset: specific GTPase values re-calculated from previous work by Sossong et al. [10]. GTPase activity values shown in their Fig. 1 were converted into specific activity values, by dividing them by the FtsZ concentration, and adapted to a relative scale.

GTPase activity values were also measured over a similar range of FtsZ concentration (Fig. 3, insert). The two series of values exhibit a remarkably similar relative variation.

3.4. Molecular dynamics of simulated FtsZ depolymerization

To provide more specific data regarding the relationship between the position of the interface and its active/inactive state, an experiment was designed in which, after the 8 ns MD trajectory, the FtsZ pentamer was shortened to tetramer by removing the lower monomer (Fig. 4A) and then subjected to an additional 8 ns of unrestricted MD. In the new structure, the interface V_A was no longer present and the interfaces V_B and V_D (now IV′$_B$ and IV′$_D$) remained in their respective positions and also exhibited the same behavior as in the pentamer. In the interface IV′$_B$, the initial catalytic water molecule leaves the active centre after 6 ns being rapidly replaced by a second water molecule that adopts the correct distance and angle, thus maintaining the active conformation. Results are summarized in Table 1 and detailed in Supplementary Figs. S1 and S2.

A more dramatic effect was observed at interface V_C (now IV′$_C$) which experimented a change in its position from centred to close-to-end: starting from an inactive state (Fig. 4B, interface V_C), a water molecule localized in the proximity of P_γ adopted the correct distance and angle within the first 4 ns of the additional trajectory (dark green line in Fig. 4B, interface IV′$_C$), maintaining its new stable position until the end of the MD. Thus, the internal structure of an inactive interface switched to active simply due to the change in its relative position from a central to a close-to-end location.

In conclusion, our analysis of the different ways in which interfaces in FtsZ polymers behave indicates that GTPase activity may be favoured at the end of the polymers, supporting one putative hypothesis of FtsZ filaments with independent GTP sites recently proposed based on kinetic measurement of GTPase activity [14].

These results raise some questions on the in vivo function of FtsZ, in particular on the connection between the hydrolytic reaction, the filament dynamics and the mechanical properties of the polymer. If the GTPase activity is restricted to filament ends, then polymer bending caused by a putative GTP hydrolysis along the entire polymer cannot be the force generation mechanism. Another

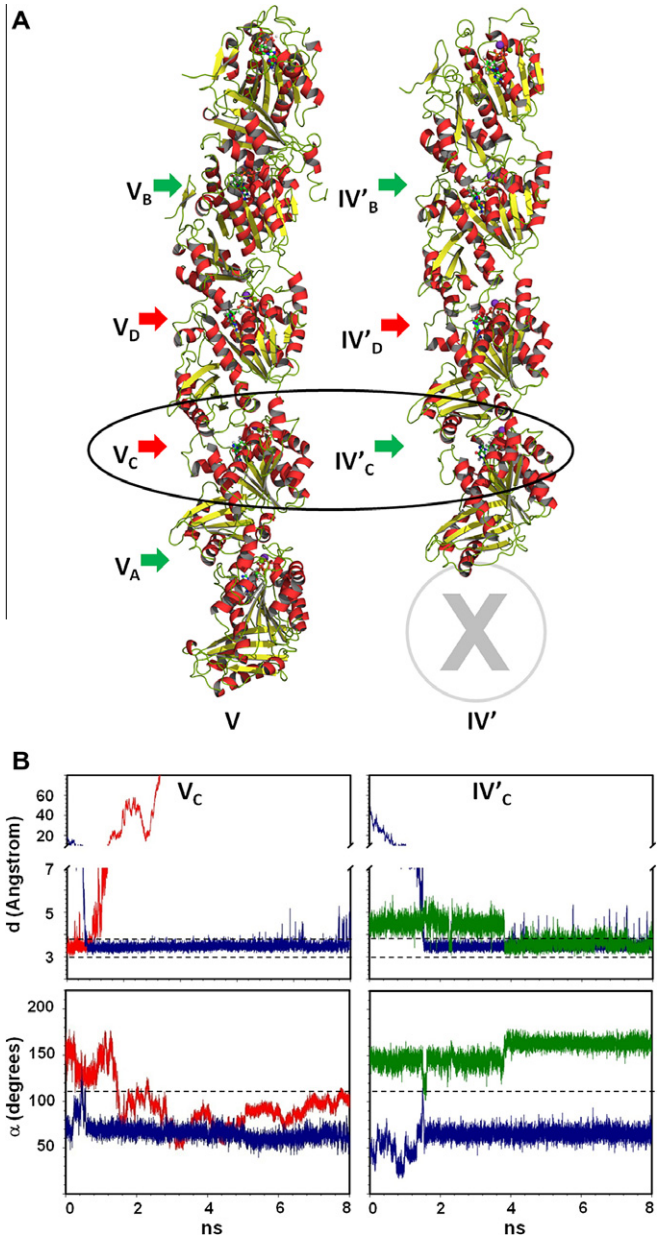

Fig. 4. (A) Representation of modelled FtsZ pentamer (V) and tetramer (IV′) after subtracting of one FtsZ monomer. Transition from inactive to active state in the case of interfaces V_C to IV′$_C$ is highlighted. (B) Continuous measurement of "*d*" distance and "α" angle for all water molecules present in the interfaces V_C and IV′$_C$ over 8 ns of unrestricted MD. Labels and colours are as in Fig. 2.

important extrapolation of this model is the reduction of the control point to only the two ends. Two discrete points per filament seem easier to manage than a controlling mechanism extending along all the filament subunits.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.03.042.

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