Comparative Analyses of Gelsolin and the Gelsolin Homology Domains of Flightless-I

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ABSTRACT

Flightless-I is a unique member of the gelsolin superfamily alloying six gelsolin homology domains and leucine-rich repeats. Flightless-I is an established regulator of the actin cytoskeleton, however, its biochemical activities in actin dynamics are still largely elusive. To better understand the background of the biological functioning of Flightless-I we studied the actin activities of Drosophila Flightless-I by in vitro bulk fluorescence spectroscopy and single filament fluorescence microscopy. Flightless-I inhibits polymerization by high-affinity (~nM) filament barbed end capping, moderately facilitates nucleation by low-affinity (~ µM) monomer binding and does not sever actin filaments.

Flightless-I was found to interact with actin and affect actin dynamics in a calcium-independent fashion in vitro, suggesting the lack of calcium-mediated activation and conformational change of protein. For the comparative structural analysis of the six gelsolin homology domains (GH16) of gelsolin and Flightless-I, we used a combination of biophysical and biochemical approaches. The use of external (8-anilinonaphthalene-1-sulfonic acid; ANS) fluorophores revealed that calcium-binding induces structural changes in gelsolin but the conformational behavior of Flightless-I GH16 was not significantly affected by the divalent cation. Our experimental findings are supported by bioinformatics analysis predicting that the sequence elements responsible for Ca^{2+}-activation of GSN are not conserved in Flightless-I GH16 and its structure is similarly rigid and organized as in case of gelsolin.

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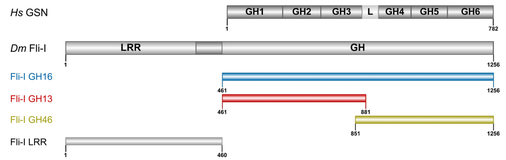
DOMAIN ORGANIZATION OF GELSOLIN AND FLIGHTLESS-I



Ca^{2+} -activation of gelsolin

Gelsolin (GSN) the eponymous member of the gelsolin-homology protein family possesses Ca^{2+}-dependent actin activities, including nucleation, capping and severing. Ca^{2+}-activation of GSN is coupled to both local and global conformational changes of the molecule. The binding of calcium ions activates gelsolin (GSN) by opening the three latches that stabilize the inactive structure. Early in this process, the tail latch must be released, exposing the F-actin side-binding site on G2 and allowing the G3–G4 linker to adopt an extended conformation that enables the two halves of GSN to separate from each other. Subsequent opening of the G1–G3 and G4–G6 latches entails dramatic rearrangement of the relative positions of the domains. While comparison of the structures of the activated halves of gelsolin with those in inactive gelsolin defines the beginning and end points of the activation process, its mechanism remains largely speculative.

AIMS

- ❑ WHAT IS THE ROLE OF FLIGHTLESS-I IN ACTIN DYNAMICS?
- ❑ WHAT ARE THE CONFORMATIONAL CHARACTERISTICS OF FLIGHTLESS-I?

CONCLUSION

- ❑ IN CONTRAST TO GELSOLIN, FLIGHTLESS-I POSSESSES Ca^{2+}-INDEPENDENT INTERACTIONS AND ACTIVITIES IN THE REGULATION OF ACTIN DYNAMICS.
- ❑ GELSOLIN AND FLIGHTLESS-I HAVE DIFFERENT CONFORMATIONAL CHARACTERISTICS AS REVEALED BY DIFFERENT METHODS. Ca^{2+}-INDUCES PRONOUNCED STRUCTURAL REARRANGEMENTS IN GELSOLIN, WHILE IT DOES NOT INFLUENCE SIGNIFICANTLY THE CONFORMATION OF FLIGHTLESS-I.
- ❑ OUR WORK IDENTIFIES THE GH13 DOMAINS AS THE MAIN ACTIN INTERACTING REGION OF FLI-I, SINCE NEITHER THE C-TERMINAL GH46 DOMAINS NOR THE LEUCINE-RICH REPEAT REGION AT THE N TERMINUS ASSOCIATES TO ACTIN. IN THE PRESENCE OF PROFILIN FLI-I IS ONLY ABLE TO CAP ACTIN FILAMENT BARBED ENDS BUT FAILS TO PROMOTE ACTIN ASSEMBLY FROM PROFILIN:ACTIN .

RESULTS

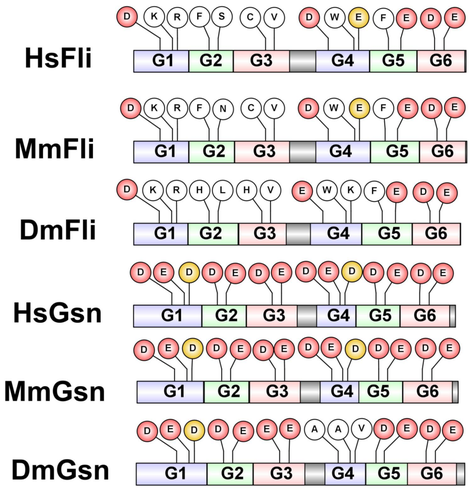


Figure 1. Comparative analysis of the calcium binding sites within the gelsolin homology domains of Fli-I and gelsolin from different species

UniProt IDs: Q13045 (Hs Fli-I), Q9JJ28 (Mm Fli-I), Q24020 (Dm Fli-I), P06396 (Hs GSN), P13020 (Mm GSN), Q07171 (Dm GSN). The analysis was performed by ClustalX. Hs, Homo sapiens; Mm, Mus musculus; Dm, Drosophila melanogaster; FliI, Flightless-I; GSN, gelsolin; G, gelsolin homology. Red: Conserved type-II Ca^{2+}-binding site, Yellow: Conserved type-I Ca^{2+}-binding site, Hollow: not-conserved Ca^{2+}-binding site

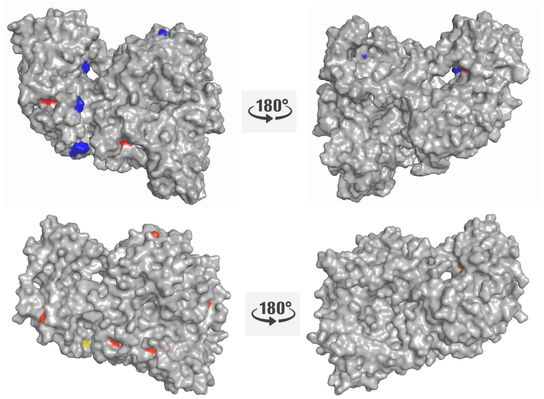
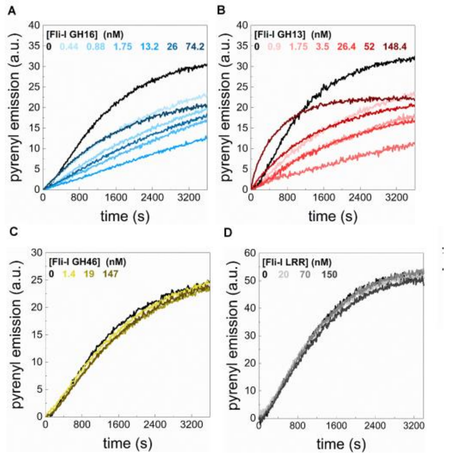


Figure 2. Surface of GSN (A-B) and Fli-I (C-D)

Surface of GSN (RCSB PDB code: 3FFN) and FliI GH16 (Uniprot ID: Q24020, using I-TASSER). Red: Conserved type-II Ca^{2+}-binding site, Yellow: Conserved type-I Ca^{2+}-binding site, Blue: not-conserved Ca^{2+}-binding site. The most of the Ca^{2+}-binding sites are partly or totally buried, especially in case of GSN. Total surface of binding sites of Fli-I GH16: 383.3 Å², of GSN: 134.7 Å².



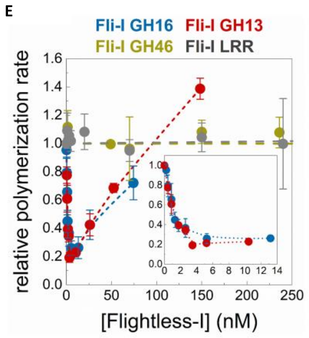


Figure 3. The gelsolin homology domains of Flightless-I affect actin assembly that relies on its GH13 domains.

(A–D) Representative pyrenyl fluorescence emission kinetics recorded in the absence or presence of different concentrations of GST-Fli-I constructs. Conditions: 2.5 µM actin (5% pyrenyl labeled). (E) Relative polymerization rate as a function of [GST-Fli-I]. Data are shown as mean ± SD, n = 2–7. Inset: enlarged view of the data corresponding to low [GST-Fli-I] (<14 nM).

IN CONTRAST TO GELSOLIN THE GH DOMAINS OF Fli-I INFLUENCE ACTIN DYNAMICS IN A Ca^{2+}-INDEPENDENT MANNER

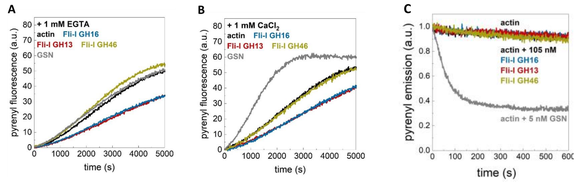


Figure 4.

(A-B) Representative pyrenyl emission kinetics recorded in the absence or presence of Fli-I constructs (as indicated) and gelsolin (GSN) and in the absence or presence of 1 mM EGTA (A) or 1 mM $CaCl_2$ (B). Conditions: 2.5 µM actin (5 % pyrenyl labelled). (C) Kinetics of actin polymer disassembly as followed by the decrease in pyrenyl fluorescence emission in the absence or presence of GST-Fli-I or GSN. Conditions: [actin] = 50 nM (50 % pyrenyl labeled), [GSN] = 5 nM, [GST-Fli-I] = 105 nM, [$CaCl_2$] = 1 mM.

SEQUENCE BASED IN SILICO ANALYSIS OF GSN AND Fli-I

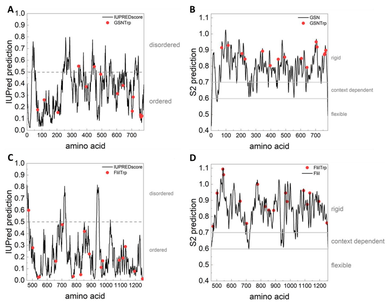


Figure 5.

Sequence based in silico analysis of GSN and Fli-I.

(A-D) Bioinformatic analysis of primary amino acid sequence of GH16 domains of GSN (A-B) and Fli-I (C-D). IUPred scores indicates almost entirely characterized low disorder probability of both protein's sequence with ordered structural segment dispositions of Trp residues (A, C). DynaMine (S2) prediction tendency represents similar protein backbone dynamics (B, D).

SURFACE HYDROPHOBICITY ASSESSMENT WITH 1-ANILINO-8-NAPHTALENE SULFONATE

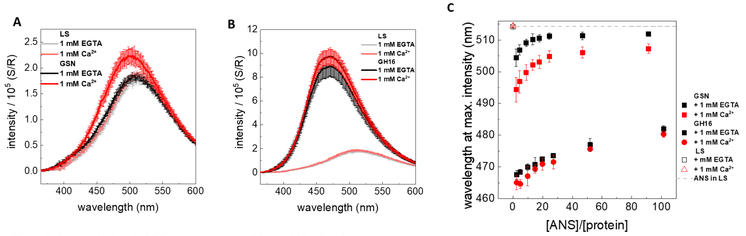


Figure 6. Surface hydrophobicity assessment of GSN and Fli-I GH16.

(A-B) Emission spectra of 13.1 µM ANS (1-anilino-8-naphtalene sulfonate) beside 0.98 µM GSN (A) and 0.88 µM Fli-GH16 (B) in presence and absence of $CaCl_2$. $CaCl_2$-free and $CaCl_2$-containing LS controls are the same for both graph. Data are shown as mean ± standard deviation, n = 3-4. (C) Wavelengths corresponding to the maximal ANS intensity during ANS titration. Initial concentrations of GSN and Fli-GH16 were 1 µM, and 0.9 µM respectively. The mean of Ca^{2+}-free and Ca^{2+}-containing controls were calculated independently of the ANS concentration and are shown as empty square or triangle, because there was no shift during titration. Mean of all controls is shown as dashed gray line for ease of comparison of shifts in protein containing samples. Data are shown as mean and 95% confidence interval ($CI_{95\%}$), $n_{protein\ containing\ samples}$ = 4; $n_{controls}$ = 24.