Activation in isolation: exposure of the actin-binding site in the C-terminal half of gelsolin does not require actin[1]

Kartik Narayan[a,c], Sakesit Chumnarnsilpa[a], Han Choe[b], Edward Irobi[a], Dunja Urosev[c], Uno Lindberg[d], Clarence E. Schutt[e], Leslie D. Burtnick[c,2], Robert C. Robinson[a,*]

[a] *Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, Box 582, 751 23 Uppsala, Sweden*
[b] *Department of Physiology, University of Ulsan College of Medicine, 388-1 Pungnapdong, Songpa-Gu, Seoul 138-736, South Korea*
[c] *Department of Chemistry and Center for Blood Research, University of British Columbia, Vancouver, BC, Canada V6T 1Z1*
[d] *Department of Cell Biology, Wenner-Gren Institute, Stockholm University, 10691 Stockholm, Sweden*
[e] *Henry H. Hoyt Labs, Department of Chemistry, Princeton University, Princeton, NJ 08544, USA*

Received 22 May 2003; accepted 1 June 2003

First published online 29 August 2003

Edited by Amy McGough

Abstract Gelsolin requires activation to carry out its severing and capping activities on F-actin. Here, we present the structure of the isolated C-terminal half of gelsolin (G4–G6) at 2.0 Å resolution in the presence of Ca^{2+} ions. This structure completes a triptych of the states of activation of G4–G6 that illuminates its role in the function of gelsolin. Activated G4–G6 displays an open conformation, with the actin-binding site on G4 fully exposed and all three type-2 Ca^{2+} sites occupied. Neither actin nor the type-1 Ca^{2+}, which normally is sandwiched between actin and G4, is required to achieve this conformation.

Key words: Gelsolin; Actin; Calcium-activation

1. Introduction

The ability of actin to cycle between monomeric and filamentous states enables dynamic rearrangement of cellular machinery in response to extracellular stimuli [1,2]. Gelsolin is one protein used by cells to regulate the state of intracellular actin (reviewed in [3] and [4]). Furthermore, cell death releases actin into blood plasma, where conditions are such that, in the absence of opposing factors, it would polymerize into long filaments that would tend to elevate plasma viscosity. To avoid accompanying complications, an actin scavenger system, comprised of an extracellular form of gelsolin and vitamin D-binding protein, has evolved [5]. Gelsolin rapidly severs actin filaments at substoichiometric concentrations, remaining as a cap on their barbed ends. Simultaneously, the low concentration of monomeric actin in plasma promotes release of monomers from the pointed ends of the filaments [6]. Vitamin D-binding protein then sequesters G-actin into a stoichiometric complex that is rapidly removed from the bloodstream in the liver.

Gelsolin comprises six analogously folded domains (G1–G6, respectively) [7] that evolved as a result of a gene triplication followed by gene duplication [8,9]. The fold characteristic of an individual gelsolin domain is found in another family of actin-binding proteins that shares limited similarity at the amino acid sequence level, the ADF/cofilin family [10,11]. Fully assembled, plasma gelsolin is a 755-residue protein in which long, flexible peptide linkers connect the well-defined globular domains. Three-dimensional evidence of the gene replication events is manifested in the generally similar appearances of the first and second halves of gelsolin, particularly when comparisons are made of the pairs of domains, G1 with G4, G2 with G5, and G3 with G6, respectively [7]. In the presence of Ca^{2+}, gelsolin adopts an activated state in which three previously masked actin-binding sites are exposed. These include actin monomer-binding sites on G1 and G4, and a filament side-binding site on G2 [12,13].

The structure of the C-terminal half of gelsolin in a complex with G-actin has been solved previously and refined to 3.0 Å resolution [14,15]. We present here the structure of the C-terminal half of gelsolin, crystallized in the presence of Ca^{2+}, but in the absence of actin. Interaction with actin is not a prerequisite for attaining the activated conformation.

2. Materials and methods

G-actin was prepared from an acetone powder of rabbit skeletal muscle powder [16] and further fractionated by gel filtration on Sephacryl S-300 (Amersham Biosciences). The gene fragment coding for gelsolin residues 414–742 (G4–G6) was engineered into a modified PGex-6P-1 plasmid (Amersham Biosciences) using polymerase chain reaction. The plasmid was modified to encode an eight-histidine tag, followed by a thrombin cleavage site, ahead of the N-terminus of G4–G6. DNA sequencing with an ABI model 310 DNA sequencer verified the identity of the construct.

G4–G6 was expressed in *Escherichia coli* XL-1 Blue cells grown in LB containing 100 μg/ml ampicillin and induced with 0.5 mM IPTG for 3 h at 30°C. Cells were lysed by sonication, and the suspension clarified by centrifugation at 20 000×*g* for 30 min at 4°C. 3.0 ml of

*Corresponding author. Fax: (46)-18-4714975; Website: http://www.imbim.uu.se/forskning/robinsonresearch.html.
E-mail addresses: kartik@princeton.edu (K. Narayan), sakesit@hotmail.com (S. Chumnarnsilpa), hchoe@amc.seoul.kr (H. Choe), edwardirobi@hotmail.com (E. Irobi), dunja_urosev@hotmail.com (D. Urosev), uno@cellbio.su.se (U. Lindberg), schutt@princeton.edu (C.E. Schutt), burtnick@chem.ubc.ca (L.D. Burtnick), bob.robinson@imbim.uu.se (R.C. Robinson).

[1] Data deposition: The atomic coordinates and merged structure factors have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID code 1P8X).
[2] Website: http://www.chem.ubc.ca/personnel/faculty/burtnick.

Abbreviations: G4–G6, gelsolin domains 4–6, respectively

0014-5793/03/$22.00 © 2003 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)00933-5

Table 1
Data collection and refinement statistics

Wavelength (Å)	1.068
Space group	$P2_12_12_1$
Unit cell	$a=84.7$, $b=90.1$, $c=156.9$ Å, $\alpha=\beta=\gamma=90°$
Resolution range (Å)	20.0–2.0 (2.1–2.0)
Total reflections	512 059 (70 689)
Unique reflections	81 129 (11 619)
Redundancy	6.3 (6.1)
Completeness (%)	99.2 (98.7)
Average I/σ	6.6 (6.0)
R_{merge}[a] (%)	5.4 (13.0)
R_{cryst}[b] (%)	20.5 (21.6)
R_{free}[c] (%)	24.5 (27.6)
Non-hydrogen atoms (calcium ions, water)	8914 (9, 1042)
Molecule 1 consists of residues	412–635, 638–645, 656–707, 710–741
Molecule 2 consists of residues	414–455, 459–741
Molecule 3 consists of residues	414–455, 460–741
Mean temperature factor for each molecule (Å^2)	29.9, 29.1, 34.0
RMS deviation bonds (Å)	0.013
RMS deviation angles (°)	1.70

[a] R_{merge} $(\Sigma|I-\langle I\rangle|/\Sigma\langle I\rangle)$.
[b] R_{cryst} $(\Sigma||F_o|-|F_c||/\Sigma|F_o|)$.
[c] Based on 5% of the data.

Ni-NTA beads was added to the supernatant and mixed continuously for 30 min at 4°C. The beads were spun down at 1000×*g* at 4°C for 5 min, and resuspended in 300 mM NaCl, 20 mM imidazole, 10 mM Tris–HCl, pH 8.0. After washing the beads, G4–G6 was eluted by raising the imidazole concentration to 250 mM. The His tag was cleaved by addition of thrombin, with overnight dialysis against 2 mM Tris–HCl, 0.2 mM ATP, 0.2 mM $CaCl_2$, 1 mM NaN_3, 0.5 mM DTT, pH 7.6. Benzamidine Sepharose beads (Amersham Biosciences) were added to remove thrombin, and spun down at 1000×*g* at 4°C for 5 min. The supernatant was passed through a Ni-NTA column, and the flowthrough was shown to be enriched in G4–G6 by SDS–PAGE [17]. G4–G6 was further purified by size-exclusion chromatography on Superdex 200 (90×2.5 cm), equilibrated with 150 mM NaCl, 10 mM Tris–HCl, pH 8.0. To ensure activation, the sample was made 1 mM in $CaCl_2$.

Crystals of G4–G6 were obtained in similar conditions as for the G4–G6 complex with actin [14], by mixing a 10 mg/ml solution of protein with a precipitant solution consisting of 15% (w/v) PEG 8000, 100 mM Tris–HCl, pH 7.5 at 4°C, using the hanging drop vapor diffusion method. The crystals were frozen in liquid nitrogen after a 12 h soak in the precipitant solution supplemented with 20% glycerol. X-ray diffraction data were collected at 100 K on beamline 14-4 at the European Synchrotron Radiation Facility in Grenoble (Table 1). Data processing, molecular replacement (using the G4–G6 portion of PDB entry 1H1V), and refinement were carried out using the CNS [18] and CCP4 [19] suites of crystallographic programs. Initially the three molecules were refined using non-crystallographic constraints, and subsequently using non-crystallographic restraints, as differences in electron density became evident. In the final rounds of refinement, the non-crystallographic restraints were discarded, as this strategy produced an improvement in the free *R*-factor. The models were rebuilt in O [20], and superposition of molecules was achieved using LSQMAN [21]. The final model was analyzed using PROCHECK [19]. Trp677 in each molecule in the asymmetric unit is the only non-Gly residue found in the disallowed region of a Ramachandran plot. Figures were generated with the program MOLSCRIPT [22].

3. Results and discussion

3.1. Activation does not require actin

G4 and G6 in inactive gelsolin share an extended β-sheet through their cores (Fig. 1a) [7]. The structure of G4–G6 in the presence of Ca^{2+} at 2.0 Å resolution (Fig. 1b) confirms

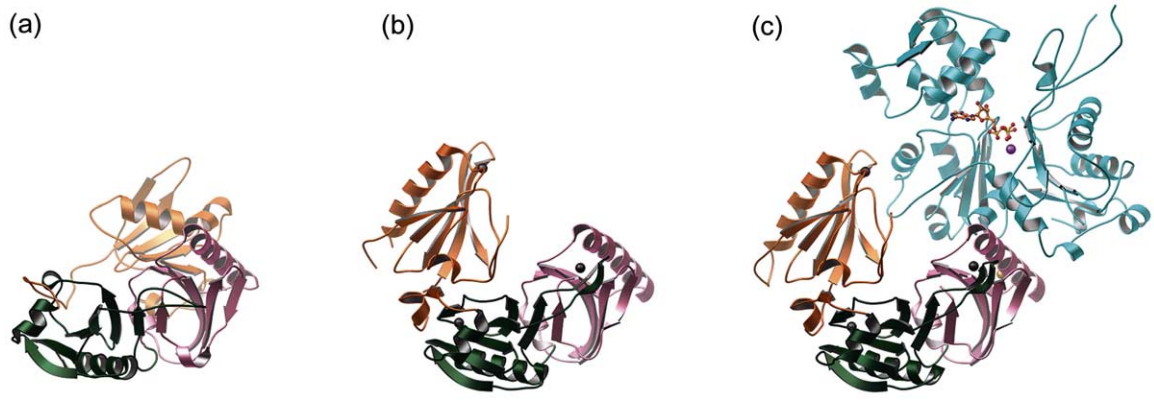

Fig. 1. Triptych of states of activation of gelsolin domains G4–G6. The ribbon representation of G4 is pink, that of G5 is green, and that of G6 is orange. The orientation of G4 is preserved in the three images. a: Domains G4–G6 have been excised from the structure of inactive gelsolin [7]. b: The novel structure at 2.0 Å resolution of G4–G6 crystallized in the presence of Ca^{2+} ions (shown as gray spheres). c: The structure at 3.0 Å resolution of the G4–G6/actin complex [15]. ATP is shown in a ball-and-stick representation and metal ions are depicted as spheres.

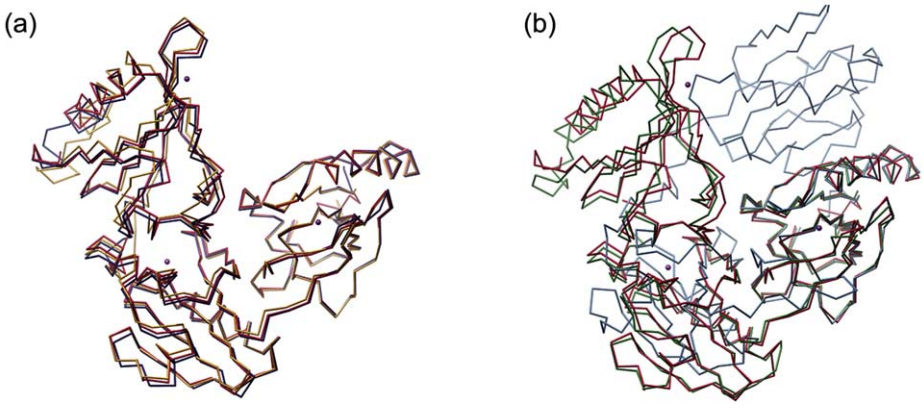

Fig. 2. Superposition of G4–G6 structures. a: The three G4–G6 molecules in the asymmetric unit superimposed on G4 reveal small angular differences among the arrangements of the domains (320 C_α atom equivalencies, RMS distance of 0.62 Å, for overlay of molecule A (red) on molecule B (gold); 312 C_α atom equivalencies, RMS distance of 1.01 Å, for overlay of molecule A on molecule C (light blue); 322 C_α atom equivalencies, RMS distance of 0.872 Å, for overlay of molecule B on molecule C). b: Structures solved in the presence and absence of actin are very similar. The Ca^{2+}-activated actin-free C-terminal half of gelsolin (red), and the C-terminal halves of gelsolin excised from the inactive (light blue) and Ca^{2+}-activated actin-bound (green) structures are superimposed on G4. The RMS C_α atom distances calculated for the three molecules in the asymmetric unit versus the activated actin-bound G4–G6 are: 0.872 Å, based on 311 C_α atom equivalencies for molecule A; 0.884 Å, based on 313 C_α atom equivalencies for molecule B; and 0.912 Å, based on 312 C_α atom equivalencies for molecule C. These indicate excellent agreement between the two forms of the activated structure. Dramatic repositioning of G6 away from its location in the inactive form is required to expose the actin-binding surface on G4.

that activation results in a large-scale displacement of G6 away from G4, which now presents a fully exposed actin-binding surface. Furthermore, the active structure, characterized by the torn sheet, displaced domains, and straightened H1 helix of G6, is essentially identical to that observed for the complex of G4–G6 with G-actin (Fig. 1c), being no more different from each other than the three molecules of the asymmetric unit of the G4–G6 crystals (Fig. 2). Hence, the activated state of G4–G6 is fully attainable in the absence of actin.

3.2. Calcium-binding sites

Gelsolin fragments bound to actin display two types of Ca^{2+}-binding sites [14,15]. Type-1 sites are found in G1 and G4, each of which shares coordination of one Ca^{2+} with actin. Here, a Ca^{2+} ion mediates the interface between actin and an actin monomer-binding domain of gelsolin. In the absence of actin, the type-1 binding site on G4–G6 remains vacant. Asp487 from G4, which would participate in coordination of the bound cation, instead forms hydrogen bonds with water in two of the three molecules in the asymmetric unit, and interacts across a crystal-packing interface with Arg596 from a symmetry-related molecule in the third.

A type-2 calcium-binding site is entirely contained within each of gelsolin domains G1–G6 [15]. These sites, having a wide spectrum of affinities for Ca^{2+}, are thought to induce conformational changes that are part of the activation process. As expected from the structure of activated G4–G6 bound to actin, each type-2 site in activated G4–G6 hosts a Ca^{2+} ion (Fig. 1b). The enhanced resolution of the present data compared to that for the complex of G4–G6 with actin permits a more complete description of the ligands within the coordination sphere of each Ca^{2+} (Fig. 3 and Table 2). Occupation of type-2 sites by Ca^{2+} facilitates disruption of interactions between gelsolin domains to release latches and render the activated gelsolin able to bind actin.

Subsequent to the submission of this work for publication, a 3.0 Å resolution structure for Ca^{2+}-activated, actin-free

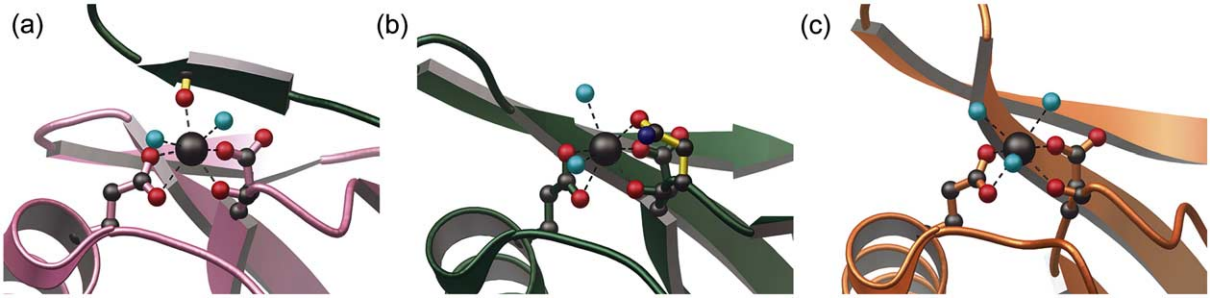

Fig. 3. Type-2 calcium-binding sites. These sites are entirely contained within the gelsolin molecule and are thought to activate the molecule both by disrupting pre-existing interactions between domains and by stabilizing new ones. Each such site is comprised of conserved interactions between calcium and a glutamic acid in Helix H1, an aspartic acid one residue prior to the C strand, and a carbonyl oxygen immediately preceding the aspartic acid (Table 2). Completion of the coordination is by water (blue spheres). Differences in coordination among the domains are: in G4, a carbonyl oxygen from G5 residue 524 participates (panel a, yellow); in G5, a side chain oxygen atom of Asn564 participates (panel b, yellow); and in G6, a third water completes the coordination (panel c).

Table 2
Coordination of Ca^{2+} at type-2 sites in the three molecules of the asymmetric unit

Domain	Residue	Atom	Distance in molecule (Å)		
			A	B	C
G4	Gly444	O	2.42	2.37	2.69
	Asp445	OD1	2.41	2.38	2.27
	Glu475	OE1	2.53	2.48	2.59
	Glu475	OE2	2.71	2.66	2.70
	Thr524	O	2.48	2.42	2.70
	Water		2.60	2.48	2.73
	Water		2.51	2.50	2.84
B-factor (Å^2)			24.0	22.6	40.4
G5	Asn564	O	2.46	2.41	2.50
	Asn564	OD1	2.47	2.50	2.55
	Asp565	OD1	2.45	2.41	2.43
	Glu587	OE1	2.74	2.67	2.79
	Glu587	OE2	2.50	2.40	2.40
	Water		2.58	2.59	2.55
	Water		2.51	2.63	2.56
B-factor (Å^2)			19.5	21.6	25.7
G6	Asp669	O	2.28	2.45	2.40
	Asp670	OD2	2.60	2.46	2.48
	Glu692	OE1	2.63	2.53	2.62
	Glu692	OE2	2.44	2.49	2.55
	Water		2.66	2.66	2.72
	Water		2.61	2.56	2.71
	Water		2.67	2.66	2.69
B-factor (Å^2)			29.4	28.8	35.6

G4–G6 appeared in print [23]. That structure was of cloned murine G4–G6, which crystallized in space group $P4_12_12$ with one protein molecule per asymmetric unit. This structure matched that previously reported for activated G4–G6 bound to actin and matches the structures reported here, except that its type-2 Ca^{2+}-binding site in G4 was unoccupied. That anomaly prompted examination of the 3.0 Å data to determine whether the difference was genuine or a function of the lower resolution of the structure determination. The F_o–F_c electron density difference map calculated from the deposited coordinates and structure factors for PDB entry 1NPH reveals a 6.5σ peak at the expected position of the G4 type-2 Ca^{2+}. In addition, the type-2 coordinating oxygens at this site have released protein–protein polar contacts to adopt their Ca^{2+}-bound positions. Hence, in the range of Ca^{2+} concentrations used in both crystallographic experiments (0.1–1.0 mM), G4–G6 adopts an activated form that includes three Ca^{2+} ions.

Acknowledgements: We thank Terese Bergfors for crystalliztion facilities. We acknowledge the ESRF for provision of synchrotron radiation facilities and thank Raimond Ravelli for assistance in using beamline 14-4. For financial support, we thank the Swedish Natural Science Research Council (R.C.R. and U.L.), and the Heart and Stroke Foundation of BC and the Yukon (L.D.B.). K.N. thanks the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) for support through a grant to U.L.

References

[1] Oosawa, F., Asakura, S., Hotta, K., Imai, N. and Ooi, T. (1959) J. Polymer Sci. 37, 323–336.
[2] Small, J.V., Stradal, T., Vignal, E. and Rottner, K. (2002) Trends Cell Biol. 12, 112–120.
[3] Sun, H.Q., Yamamoto, M., Mejillano, M. and Yin, H.L. (1999) J. Biol.Chem. 274, 33179–33182.
[4] dos Remedios, C.G., Chhabra, D., Kekic, M., Dedova, I.V., Tsubakihara, M., Berry, D.A. and Nosworthy, N.J. (2003) Physiol. Rev. 83, 433–473.
[5] Herrmannsdoerfer, A.J. et al. (1993) Am. J. Physiol. 265, G1071–G1081.
[6] Yin, H.L. and Stossel, T.P. (1979) Nature 281, 583–586.
[7] Burtnick, L.D., Koepf, E.K., Grimes, J., Jones, E.Y., Stuart, D.I., McLaughlin, P.J. and Robinson, R.C. (1997) Cell 90, 661–670.
[8] Kwiatkowski, D.J., Stossel, T.P., Orkin, S.H., Mole, J.E., Colten, H.R. and Yin, H.L. (1986) Nature 323, 455–458.
[9] Way, M. and Weeds, A. (1988) J. Mol. Biol. 203, 1127–1133.
[10] Hatanaka, H., Ogura, K., Moriyama, K., Ichikawa, S., Yahara, I. and Inagaki, F. (1996) Cell 85, 1047–1055.
[11] Van Troys, M., Dewitte, D., Verschelde, J.L., Goethals, M., Vandekerckhove, J. and Ampe, C. (1997) J. Biol. Chem. 272, 32750–32758.
[12] Way, M., Gooch, J., Pope, B. and Weeds, A.G. (1989) J. Cell Biol. 109, 593–605.
[13] Pope, B., Way, M. and Weeds, A.G. (1991) FEBS Lett. 280, 70–74.
[14] Robinson, R.C., Mejillano, M., Le, V.P., Burtnick, L.D., Yin, H.L. and Choe, S. (1999) Science 286, 1939–1942.
[15] Choe, H., Burtnick, L.D., Mejillano, M., Yin, H.L., Robinson, R.C. and Choe, S. (2002) J. Mol. Biol. 324, 691–702.
[16] Spudich, J.A. and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871.
[17] Laemmli, U.K. (1970) Nature 227, 680–685.
[18] Brunger, A.T. et al. (1998) Acta Crystallogr. D 54, 905–921.
[19] CCP4 (1994) Acta Crystallogr. D 50, 760–763.
[20] Jones, T.A., Zou, J.-Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Crystallogr. A 47, 110–119.
[21] Kleywegt, G.J. (1996) Acta Crystallogr. D 52, 842–857.
[22] Kraulis, P. (1991) J. Appl. Cryst. 24, 946–950.
[23] Kolappan, S., Gooch, J.T., Weeds, A.G. and McLaughlin, P.J. (2003) J. Mol. Biol. 329, 85–92.