Global Structure Changes Associated with Ca$^{2+}$ Activation of Full-length Human Plasma Gelsolin

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Gelsolin regulates the dynamic assembly and disassembly of the actin-based cytoskeleton in non-muscle cells and clears the circulation of filaments released following cell death. Gelsolin is a six-domain (G1–G6) protein activated by calcium via a multi-step process that involves unfolding from a compact form to a more open form in which the three actin-binding sites (on the G1, G2, and G4 subdomains) become exposed. To follow the global structural changes that accompany calcium activation of gelsolin, small-angle x-ray scattering (SAXS) data were collected for full-length human plasma gelsolin at nanomolar to millimolar concentrations of free Ca$^{2+}$. Analysis of these data showed that, upon increasing free Ca$^{2+}$ levels, the radius of gyration ($R_g$) increased nearly 12 Å, from 31.1 ± 0.3 to 43 ± 2 Å, and the maximum linear dimension ($D_{max}$) of the gelsolin molecule increased 55 Å, from 100 to 155 Å. Structural reconstruction of gelsolin from these data provided a striking visual tracking of the gradual Ca$^{2+}$-induced opening of the gelsolin molecule and highlighted the critical role played by the flexible linkers between homologous domains. The tightly packed architecture of calcium-free gelsolin, seen from both SAXS and x-ray crystallographic models, is already partially opened up in as low as 0.5 mM Ca$^{2+}$. Our data confirm that, although the molecule springs open from 0 to 1 μM free Ca$^{2+}$, even higher calcium concentrations help to stabilize a more open structure, with increases in $R_g$ and $D_{max}$ of ~2 and ~15 Å, respectively. At these higher calcium levels, the SAXS-based models provide a molecular shape that is compatible with that of the crystal structures solved for Ca$^{2+}$/gelsolin C-terminal and N-terminal halves ± monomeric G-actin. Placement of these crystal structures within the boundaries of the SAXS-based model suggests a movement of the G1/G2 subunits that would be required upon binding to actin.

Gelsolin, an ~80-kDa globular protein, exists as a cytoplasmic as well as a plasma isoform and is encoded by a single gene on chromosome 9 (1, 2). Gelsolin functions to regulate the dynamic assembly and disassembly of the actin-based cytoskeleton by severing F-actin and remaining capped at the fast-growing end of the filament. In addition, gelsolin also initiates formation of F-actin by binding to two monomeric G-actin molecules. In vivo studies on gelsolin, particularly using gelsolin-null mice and/or fibroblasts, have identified its critical role in many processes involving cell motility or cortical shape changes (for recent reviews, see Refs. 3 and 4). These physiological activities of gelsolin are predominantly regulated by Ca$^{2+}$ levels and phosphoinositides (reviewed in Ref. 5).

Gelsolin consists of six homologous subdomains (designated G1–G6) (6) that appear to have evolved through a gene triplication process followed by an additional duplication (7–10). Hence, gelsolin has been considered as two domain triplets of the N- and C-terminal halves (G1–G3 and G4–G6, respectively) separated by a g3–g4 linker sequence that makes up ~8% of the total molecular mass, or ~70 amino acids long (6). Both the cytoplasmic and plasma isoforms have nanomolar and micromolar affinity Ca$^{2+}$-binding sites (7, 11).

The crystal structure of the full-length horse plasma gelsolin in the Ca$^{2+}$-free inactive form (Protein Data Bank code 1D0N) confirms that the six subdomains are structurally homologous and fold together to adopt a compact globular shape (6). In this structure, the two halves of gelsolin are held together by extensive interactions between a C-terminal helical extension of the G6 subdomain (often referred to as the C-terminal latch) and the F-actin-binding helix found in the G2 subdomain. As expected, the actin-binding regions of the G1, G2, and G4 subdomains are buried. It was proposed that Ca$^{2+}$ binding disrupts the interactions between the N- and C-terminal halves, enabling each half to bind actin relatively independently (6).

To date, no high resolution structure of full-length plasma gelsolin, either fully or partially activated by Ca$^{2+}$, has been reported. Most of the structural interpretation at atomic detail regarding a Ca$^{2+}$-activated structure of gelsolin has been derived from crystal structures of Ca$^{2+}$-bound N- and C-terminal halves of gelsolin: the Ca$^{2+}$-activated C-terminal half of gelsolin in both the presence (Protein Data Bank code 1H1V) (12) and absence (Protein Data Bank codes 1NP8 and 1PX8) (13, 14) of actin and the Ca$^{2+}$-activated N-terminal half of gelsolin bound to actin (15). In both the absence and presence of Ca$^{2+}$ ions, the basic architecture of the six homologous domains is a central five-stranded mixed β-sheet sandwiched...
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by two α-helices, one longer one running parallel to and another shorter one running almost perpendicular to the β-sheets. Based on the Ca$^{2+}$-activated structures, it has been inferred that, although intradomain tertiary structural content remains almost unchanged, a rearrangement in the relative orientation of the six domains is induced upon chelating to Ca$^{2+}$ ions. The similarity between the crystal structures of the Ca$^{2+}$-bound C-terminal half of gelsolin in the presence and absence of actin provides evidence that Ca$^{2+}$ ions alone can induce the actin-binding "open" form of this half of the molecule (14).

Additional structural information on the conformational dynamics of ligand-induced structural changes in full-length gelsolin has been provided by dynamic light scattering (17, 18), circular dichroism (18), and radiolytic footprinting (11, 19) studies. Dynamic light scattering results revealed that the hydrodynamic radius ($R_h$) of gelsolin increases from 3.9 to 4.5 nm upon addition of Ca$^{2+}$ up to 1 mM to an EGTA-containing solution (18). This observed increase in $R_h$ was shown to be reversible from 10 μM Ca$^{2+}$ upon chelation with added EGTA. These dynamic light scattering studies concluded that, in solution, the compact Ca$^{2+}$-free gelsolin molecule opens upon binding Ca$^{2+}$ following a sigmoidal relationship, which is reversible and in which 50% "activation" occurs with 30 mM Ca$^{2+}$, whereas a maximum $R_h$ is reached at ~1 μM Ca$^{2+}$ (18). This observed reversibility is also supported by recent EGTA chelation studies on different crystalline forms of the Ca$^{2+}$-activated N- and C-terminal halves of gelsolin whereby the subdomains rearranged toward the compact inactive form (20).

Critical insight into the Ca$^{2+}$ activation process of full-length gelsolin in solution was provided by Kisela et al. (19), who used a radiolytic footprinting technique to probe the changes in surface-exposed residues on gelsolin as a function of free Ca$^{2+}$. This study supports a three-stage structural activation of gelsolin beginning with the Ca$^{2+}$-free state showing a shift in conformation toward the second state occurring at ~0.1 to ~5 μM Ca$^{2+}$ and a midpoint at ~0.5 μM Ca$^{2+}$, which evolves into a third structural state at ~10 μM to 1 mM Ca$^{2+}$ and a midpoint at ~100 μM Ca$^{2+}$. This three-state model explains the increased depolymerizing activity of F-actin at higher Ca$^{2+}$ levels in plasma compared with intracellular levels, which would be maintained below 10 μM free Ca$^{2+}$.

We present here the global structural changes that accompany calcium activation of full-length gelsolin from nanomolar concentrations as detected by small-angle x-ray scattering (SAXS).2 Pairwise distribution function ($P(r)$) analysis and ab initio molecular modeling of these data provide new structural insights into the Ca$^{2+}$-induced opening of the gelsolin molecule and highlight the critical role played by the flexible linkers between homologous domains.

MATERIALS AND METHODS

Protein Expression and Purification—Recombinant human plasma gelsolin was prepared according to previously described methods (21). Briefly, human plasma gelsolin cDNA was cloned into pET3a expression vectors. Expressed gelsolin was obtained by solubilizing inclusion bodies with 8 M urea, followed by purification employing anion and cation exchange chromatographies (21). Protein concentration was determined by the Bradford assay (22), and purity was assessed by SDS-PAGE. To ensure monodispersity of our SAXS sample, devoid of any higher molecular mass entity, immediately prior to the SAXS experiments, gelsolin was further purified by gel filtration chromatography using a Superdex 200 column on an LCC-501 PLUS FPLC system (Amersham Biosciences AB, Uppsala, Sweden). The protein was eluted under Ca$^{2+}$-free conditions using buffer composed of 25 mM Tris (pH 8.0), 30 mM NaCl, 1 mM EGTA, and 1 mM dithiothreitol. The anticipated retention volume for gelsolin was estimated using known protein standards. Gelsolin eluted as a single peak, which was collected and concentrated using Centricon concentrators. The final concentration was determined spectrophotometrically to be 10 mg/ml using a $A_{280} = 1.24$ (23). Four sample concentrations (10, 5, 2.5, and 1 mg/ml) were made by dilution of the 10 mg/ml stock. A limited number of Ca$^{2+}$ concentration experiments were repeated with plasma gelsolin extracted from bovine plasma (Pel-Freez Biologicals, Rogers, AR) employing a slightly modified ion exchange protocol published by Kurokawa et al. (23) and following it with an S200 gel filtration step.

Sample Preparation for SAXS Experiment—Given a final sample volume of 50 μl in the buffer conditions mentioned above, free and total calcium levels were estimated using WEBMAXC Version 2.1 (www.stanford.edu/~cappton/maxc.html). Required amount of CaCl$_2$ to obtain the desired free Ca$^{2+}$ concentration was dried in the bottom of Eppendorf tubes. These tubes were prepared in duplicate. Just prior to SAXS experiments, diluted gelsolin stock (final concentration of 2 mg/ml) was added to the tubes containing Ca$^{2+}$ and mixed thoroughly. Matched buffers (the S200 elution buffer) for each sample were prepared in a similar way. The free Ca$^{2+}$ concentration was varied from 0 to 1 mM.

SAXS Data Acquisition and Analysis—SAXS data were collected at beamline X21 at the National Synchrotron Light Source at Brookhaven National Laboratory. 50 μl of each sample and its matched buffer were exposed to x-rays for 5 min at 10°C. Scattering images were collected using a charge-coupled device detector (MarUSA Inc., Evanston, IL). The center of the beam on the detector and the sample-to-detector distance were predetermined using the scattering pattern of silver benenate powder. Images were circularly averaged, buffer-subtracted, and scaled to obtain relative scattering intensity ($I$) as a function of momentum transfer vector $q$ ($q = 4\pi\sin\theta/\lambda$).

For monodisperse globular proteins, a plot of ln($I(q)$) versus $q^2$ at low $q$, generally where $q \times R_g \leq 1$, will be linear and will fit to Equation 1.

$$I(q) = I_0e^{-(qR_g^2q^2)/3)}$$  
(Eq. 1)

Such analysis is known as Guinier analysis and provides an estimation of the scattering particle’s radius of gyration ($R_g$) and the forward or zero-angle scatter ($I_0$) from the slope ($-R_g^2/3$) and the y intercept $I_0$.

Guinier analysis of all scattering data was done using the Primus software package (24). To ensure monodispersity of the sample, the $I_0$ values derived from x-ray scattering data

2 The abbreviation used is: SAXS, small-angle x-ray scattering.
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of the gelsolin samples were compared with those of a standard protein (lysozyme) (25) measured in the same sample cell on the same day.

Indirect Fourier transformation of the scattering data over the measured $q$ range gives a pairwise distribution function of interatomic vectors ($P(r)$). The inverse Fourier transform of $I(q)$ yields $P(r)$, which is the frequency of vector lengths connecting small-volume elements within the entire volume of the scattering particle (Equation 2).

$$P(r) = \left(\frac{1}{2}\pi^2\right) \int I(q)q \cdot r \sin(q \cdot r) dq$$  (Eq. 2)

$P(r)$ goes to zero at the maximum linear dimension of the particle ($D_{max}$), $R_g$ and forward scatter ($I_0$) were calculated from the second and zeroth moments of $P(r)$, respectively. $R_g$ is defined as the root mean square of all elemental volumes from the center of mass of the particle, weighted by their scattering densities. $I_0$ is directly proportional to the molar particle concentration multiplied by the square of the scattering particle’s molecular weight for particles with the same mean scattering density. The GNOM45 software package (26) was used for all $P(r)$ analysis. For all analyses, an equal number of data points, ranging from $q$ of 0.015–0.25 Å$^{-1}$, were used.

Ab Initio Modeling—A three-dimensional scattering shape for each gelsolin sample that best fit the corresponding SAXS intensity data was generated using GASBOR22IQ software (27). Structure regeneration using no shape or symmetry bias and 800 dummy residues (packing radius of 1.9 Å each) was used to model all data sets for the Ca$^{2+}$ activation of full-length gelsolin. A Fibonacci grid order of 15 (988 water molecules) was used to add the hydration layer around the chain ensemble. Each calculation was repeated five times with comparable results, each with $\chi^2 \sim 1$ (supplemental Fig. 1). Of these, the structure with the solution with the lowest final $\chi^2$ value (see Ref. 27) of the modeled $I(q)$ profile to the raw data is presented herein. The resultant low resolution shapes for gelsolin in EGTA and in 1 mM Ca$^{2+}$ were aligned with known crystal structures of full-length gelsolin (Protein Data Bank code 1D0N) and the Ca$^{2+}$-activated halves (Protein Data Bank codes 1H1V and 1RGI) using SUPCOMP13 (28), CRYSOL26 (29) was used to estimate $R_g$, $R_h$, and scattering envelope volumes of the modeled shapes.

RESULTS AND DISCUSSION

Monodispersity of EGTA/Gelsolin Samples—Extraction of structural information on individual proteins or protein complexes in solution from scattering data requires samples that are rigorously aggregation-free. The zero-angle or forward scattering ($I_0$) is directly proportional to the mass of the scattering particle squared; therefore, it is extremely sensitive to any aggregation or high molecular weight contaminants (25). Any concentration (or time)-dependent aggregation would show an increase in $I_0$, whereas any interparticle interference effects would show a decrease in $I_0$ as a function of concentration (or time). Concentration-dependent aggregation of the EGTA/gelsolin from 1 to 10 mg/ml was determined to be negligible. Log $I(q)$ versus log $q$ plots of the 10, 5, 2.5, and 1 mg/ml gelsolin samples exhibit characteristic profiles for a compact globular scattering particle in solution (Fig. 1A). The linearity of the Guinier region of these data sets extrapolates to $I_0$ values that demonstrate no detectable increase or decrease in $I_0/c$ as a function of gelsolin concentration (Fig. 1B, inset; and Table 1).

Indirect Fourier transformation of the intensity data sets over the $q$ range of 0.015–0.25 Å$^{-1}$ was used to estimate the pairwise distance distribution curves ($P(r)$) of the scattering vectors (Fig. 1C). All four samples solved for a $D_{max}$ value equal to ~100 Å. The $I_0$ and $R_g$ values calculated from the first and second moments of the respective $P(r)$ profiles were identical, within error, for each concentration (Table 1). Together, the scattering intensity profile, Guinier approximation, and parameters from estimated $P(r)$ clearly showed that, during data acquisition, our recombinant gelsolin samples, under EGTA conditions, were monodisperse in nature.

Ca$^{2+}$ Titration of EGTA/Gelsolin Scattering Data—The increasing slope seen in intensity profiles of the gelsolin scattering data (Fig. 2A) for solutions with increasing levels of free Ca$^{2+}$ from 0.5 mM to 1 mM is indicative of a protein that is becoming larger. Each of these Ca$^{2+}$/gelsolin data sets also exhibit characteristic profiles for a compact globular scattering particle in solution. The decreasing $q$ range spanning the linear

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**TABLE 1**  
Structural parameters determined for EGTA/gelsolin samples using Guinier approximation and indirect Fourier transformation of SAXS data

| [Gelsolin] | Guinier analysis, $R_g$ | Indirect Fourier transformation, $D_{max}$, $R_g$, $I_0$, $I_0/c$ |
|-----------|------------------------|---------------------------------------------------------------|
| 10 mg/ml  | 29.4 ± 0.2              | $A$ $A$ $A$ $cm^{-1}$                                      |
| 5 mg/ml   | 29.8 ± 0.3              | $A$ $A$ $A$ $cm^{-1}$                                      |
| 2.5 mg/ml | 30.9 ± 0.7              | $A$ $A$ $A$ $cm^{-1}$                                      |
| 1 mg/ml   | 31.9 ± 1.4              | $A$ $A$ $A$ $cm^{-1}$                                      |
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Guinier regions further evidence of the increased particle size upon titration with Ca\(^{2+}\) (Fig. 2B). Analysis of the pairwise distance distribution curves estimated for these Ca\(^{2+}\)-containing samples (Fig. 2C) revealed an increase in the \(R_g\) of ~12 Å with a concomitant increase in the \(D_{max}\) values from 100 to 155 Å (Table 2). Furthermore, comparable \(I_0/\sigma\) values were calculated for these samples, indicating that the observed change in size is attributed entirely to a difference in macromolecular dimensions of the scattering particle and not due to any intermolecular aggregation events.

\textbf{Ca}^{2+} \textbf{Activation of Gelsolin}—The observed increment in the \(D_{max}\) and \(R_g\) values of recombinant human gelsolin upon binding calcium has been plotted as a function of estimated free calcium levels in Fig. 3. It is evident from this plot that there exist three distinct populations of molecular conformations within the time-averaged ensemble of structures: one at nanomolar \([\text{Ca}^{2+}]_{\text{free}}\) exhibits a significantly different average conformation than that of the EGTA structure; a second conformation, considerably more open than the EGTA structure, is observed at intermediate \([\text{Ca}^{2+}]\) levels where the \([\text{Ca}^{2+}]_{\text{free}}/\text{gelsolin}\) molar ratio is below 4:1; and a final population that represents the fully activated \([\text{Ca}^{2+}]_{\text{free}}/\text{gelsolin}\) structure (\([\text{Ca}^{2+}]_{\text{free}}/\text{gelsolin}\) \geq 6:1).

Our SAXS-based analysis suggests that an initial conformational change occurs in samples with up to ~10 nm free Ca\(^{2+}\). Assuming that the measured data at these low \([\text{Ca}^{2+}]_{\text{free}}\) represent a single predominant structure, the change from the compact EGTA structure would involve only a slight opening of the molecule, as the average structure is only 5–10 Å longer in \(D_{max}\) to 102 ± 5 Å, whereas the average overall \(R_g\) for the ensemble remains close to 30 Å. It should be noted that, because SAXS measures the time-averaged ensemble in solution, it is possible that the observed changes in SAXS at these low \([\text{Ca}^{2+}]_{\text{free}}\) is reflective not of a single predominant structure but rather of an averaging between a subset of gelsolin molecules that have been partially activated and the larger set that remain inactive. In any case, these data at nanomolar \([\text{Ca}^{2+}]_{\text{free}}\) are distinctly different from those on the calcium-chelated EGTA/gelsolin sample.

In samples immediately above 10 nm free Ca\(^{2+}\), a conspicuous change in the average conformation of gelsolin molecules is evidenced as an opening of this compact shape to ~138 Å in linear dimension and an \(R_g\) of ~40 Å. A two-step sigmoidal fit to the data suggests that this abrupt opening of the structure may begin at ~10 nm and is complete by a \([\text{Ca}^{2+}]_{\text{free}}\) ~0.1 or 1 μM. The predominant structure observed at 0.1–10 μM free Ca\(^{2+}\) (the second population) is characterized by an \(R_g\) of ~40 Å and a \(D_{max}\) just under 140 Å. Further unwinding of this structure initiates at \([\text{Ca}^{2+}]_{\text{free}}\) ~10 μM, in which gelsolin reaches its maximum open state (\([\text{Ca}^{2+}]_{\text{free}}/\text{gelsolin}\) \geq 6:1) by 200 μM with an \(R_g\) of ~45 Å and a \(D_{max}\) ~155 Å. This final population of Ca\(^{2+}\)-induced opening or activation was observed up to a

\begin{table}
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\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{pCa} & \textbf{Guinier analysis, } \(R_g\) & \textbf{\(D_{max}\)} & \textbf{\(R_g\)} & \textbf{\(I_0/\sigma\)} \\
\hline
\text{EGTA} & 30.9 ± 0.7 & 98 & 31.1 ± 0.3 & 22.2 ± 0.3 & 11.1 \\
\text{9.3} & 32.0 ± 0.9 & 108 & 31.5 ± 0.9 & 20.3 ± 0.3 & 10.2 \\
\text{8.4} & 31.1 ± 0.9 & 108 & 30.7 ± 0.5 & 20.7 ± 0.3 & 10.4 \\
\text{7.4} & 34.7 ± 0.9 & 135 & 34.6 ± 2.3 & 20.9 ± 0.6 & 10.5 \\
\text{5.7} & 38.0 ± 1.0 & 138 & 40.1 ± 1.8 & 24.1 ± 0.6 & 12.0 \\
\text{4.3} & 38.2 ± 1.1 & 140 & 42.3 ± 1.5 & 21.0 ± 0.5 & 10.5 \\
\text{4.0} & 38.0 ± 1.0 & 158 & 44.3 ± 1.2 & 22.3 ± 0.5 & 11.2 \\
\text{3.3} & 38.4 ± 1.1 & 162 & 46.1 ± 1.7 & 19.6 ± 0.6 & 9.82 \\
\text{3.0} & 37.5 ± 1.1 & 155 & 43.5 ± 1.9 & 20.6 ± 0.6 & 10.3 \\
\hline
\end{tabular}
\caption{Structural parameters determined for 2 mg/ml gelsolin samples at the indicated free pCa levels using Guinier approximation and indirect Fourier transformation of SAXS data.}
\end{table}
free calcium level of 1 mM and therefore likely represents a single predominant Ca\(^{2+}\) -saturated structure.

Consistent with the observed Ca\(^{2+}\)-dependent structural changes within recombinant human gelsolin, SAXS experiments repeated using bovine plasma gelsolin at similar protein concentrations and at calcium levels ranging from 20 nM to 40 \(\mu\)M followed a similar trend in dimensional change (Fig. 3). Further increments in [Ca\(^{2+}\)]\(_{\text{free}}\) up to 30 mM using both x-ray and neutron scattering (small-angle neutron scattering) as well as several independent experiments showed no further increments in the \(R_g\) and \(D_{\text{max}}\) values beyond those observed at [Ca\(^{2+}\)]\(_{\text{free}}\) \(\sim\) 1 mM (supplemental Figs. 2 and 3 and Table 1). Also, using bovine plasma gelsolin at 1.25 mM free Ca\(^{2+}\) and titrating back with EGTA, we have confirmed that these structural changes are reversible (data not shown).

A three-stage Ca\(^{2+}\) activation of gelsolin was first proposed by Kiselar et al. (11, 19) based upon synchrotron radiolytic footprinting experiments. Analysis of these radiolysis data revealed changes in surface-exposed amino acid residues as a function of [Ca\(^{2+}\)]\(_{\text{free}}\). Analyses of our SAXS data are consistent with this three-stage hypothesis and thus provide details on the predominant structures of the intermediate Ca\(^{2+}\)-bound states supported by this hypothesis. The Ca\(^{2+}\)-induced global conformational changes within the intact gelsolin molecule can be envisioned from shape restoration of our SAXS data.

Visual Insight into the Ca\(^{2+}\) Activation of Full-length Gelsolin—Results from \textit{ab initio} molecular modeling of the SAXS data are presented in Fig. 4. As described under “Materials and Methods,” all structures were restored employing 800 dummy residues and a grid order of 15. The “best fit” solutions of each data set, as defined by the lowest final \(\chi\) value fit between the computed \(I(q)\) of the model and the raw data (Table 3), are shown in Fig. 4. The color scheme used to depict these models is the same as that used for the SAXS data and \(P(r)\) curves shown in Fig. 2. These gelsolin models demonstrate the gradual opening of the structure as a function of decreasing pCa levels, from EGTA to 3. The SAXS data reflect the time-averaged ensemble of structural states; thus, the best fit models for the SAXS data will
display global shape changes detected from the predominant population of the molecules in solution. We modeled each SAXS data set at least five times. All SAXS-based structures were comparable between each data set in their size and shape features. The $\chi^2$ value and calculated structural parameters of each SAXS-based model are presented in Table 3. For comparison with previous dynamic light scattering results (18), Table 3 includes the model’s calculated $R_g$, $D_{\text{max}}$, $R_h$ with a 3-Å uniform

**TABLE 3**
Size and shape parameters of the best $\chi^2$ fit *ab initio* models of gelsolin SAXS data

| $p$Ca | $[\text{Ca}^{2+}]_{\text{free}}$ | Best $\chi$ value | Modeled $D_{\text{max}}$ | Modeled $R_g$ | Shell $R_h$ | Shell volume$^a$ |
|-------|-----------------|-------------------|--------------------------|---------------|-------------|-----------------|
|       | $\mu M$         |                   | $A$                      | $A$           | $A$         | $A^3$          |
| EGTA  | 0.0005          | 0.69              | 98.3                     | 30.1          | 35.2        | 95,100         |
| 8.4   | 0.004           | 1.76              | 101.6                    | 30.2          | 35.0        | 94,400         |
| 7.4   | 0.038           | 0.76              | 135.1                    | 34.4          | 37.8        | 92,900         |
| 5.7   | 2.1             | 0.81              | 143.3                    | 40.4          | 43.7        | 96,900         |
| 4.3   | 50              | 0.75              | 145.3                    | 42.5          | 41.7        | 98,100         |
| 4.0   | 100             | 0.77              | 158.3                    | 44.5          | 48.4        | 112,000        |
| 3.3   | 500             | 0.66              | 159.1                    | 45.8          | 48.7        | 104,000        |
| 3.0   | 1000            | 0.65              | 152.9                    | 44.8          | 48.1        | 109,000        |

$^a$ Specific densities for the models can be calculated by dividing the calculated shell volume by the molecular mass of gelsolin and using a partial specific density for the protein of $0.73 \text{ cc/g}$.

**FIGURE 5.** A, the best $\chi^2$ fit *ab initio* model of the SAXS data for recombinant human plasma EGTA/gelsolin shown with a space-filled dummy atom representation. B, two orthogonal ribbon representations of the crystal structure for horse plasma EGTA/gelsolin (chain A, Protein Data Bank code 1D0N) highlighting the six homologous domains, the interdomain linkers, and the C-terminal latch. C, result of an automated fit using SUPCOMB13 (28) of the high resolution EGTA/gelsolin crystal structure (ribbon with Corey-Pauling-Koltun colors) within the low resolution SAXS-based model of EGTA/gelsolin.

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FIGURE 6. The best \(\chi^2\) fit \textit{ab initio} model of the SAXS data for 1 mM free Ca\(^{2+}\)/gelsolin is shown as a space-filling dummy atom representation and overlaid (manually) with ribbon representations of the backbone structures of the Ca\(^{2+}\)-activated N-terminal (dark yellow) and C-terminal (blue) halves of gelsolin, derived from Protein Data Bank codes 1RGI and 1H1V, respectively. Three different views show that the extended activated shape of gelsolin as seen in solution is well fit on one end with the C-terminal half (G4–G6) of gelsolin and is less well accommodated by the crystal structure domain orientations reported for the N-terminal (G1–G3) domain. Superposition of the G2 and G3 domains from the crystal structure onto the SAXS-based model reorients the G1 domain considerably. The crystal structure of the Ca\(^{2+}\)-activated N-terminal domain was solved in the presence of actin; thus, this observed orientation difference suggests that the protruding G1 domain probably translocates to bind to actin, most likely via movement in the g1–g2 and/or the g3–g4 hinge.

The best-fit model of the gelsolin SAXS data under EGTA conditions has a compact globular shape of \(\sim 100\text{ Å}\) in linear dimension and an \(R_g\) of 30 Å (Fig. 5A). We used SUPCOMB13 to superimpose this model and the crystal structure of one chain from the horse plasma gelsolin dimer structure (Protein Data Bank code 1D0N) (Fig. 5B). The final overlay of the crystal structure fit well in each dimension of the SAXS-based model (Fig. 5C). Further support of this similarity is that the calculated \(I(q)\) profile from the crystal structure of the EGTA/gelsolin monomer had a \(\chi\) fit of 0.9 to the measured SAXS data.

The SAXS-based models solved at pCa levels of 9.3 and 8.4 (Fig. 4 and Table 2) would fit within Stage 1 of the Ca\(^{2+}\) activation process as proposed by Kisela\textit{r et al.} (19). Comparing these models, which are presumed to represent the predominant gelsolin conformation at these Ca\(^{2+}\) levels, with that determined for EGTA/gelsolin, one can see that there is a detectable change in the shape of the predominant molecular species even at nanomolar concentrations. In fact, the \(\chi\) fit value of the pCa 9.3 \(I(q)\) data for the crystal structure of the EGTA/gelsolin monomer was calculated at 1.3, a significant increase from the fit determined from the Ca\(^{2+}\)-free gelsolin data, thus lending further support that the average structure is indeed changing. Notably, because fluctuations of free calcium within the cell are in the nanomolar range, this less compact conformation of gelsolin better represents the physiological state than the EGTA structure. Previous studies have also reported subtle structural changes occurring in the full-length gelsolin molecule at nanomolar levels of free Ca\(^{2+}\) (7, 11). Also, opening of the C-terminal tail latch concerted with the binding of the first calcium ion has been suggested to occur under these low calcium conditions (12, 13, 18, 19, 30–34).

Increasing the free calcium level from 0.5 to 4 \(\text{nm}\) induces additional changes in the overall shape of the molecule. Modeling results suggest that changes might be occurring on both ends of the molecule, \textit{i.e.} N- and C-terminal halves, yet the \(D_{\text{max}}\) and \(R_g\) of the model computed for pCa 8.4 were nearly identical to those of the Ca\(^{2+}\)-free model. Notably, previous dynamic light scattering and CD studies (18) detected \(R_h\) and secondary structure changes at these nanomolar calcium levels, whereas the Ca\(^{2+}\) titration isotherms of radiolysis experiments were unchanged (11, 19). Even at 0.5 \(\text{nm}\) free Ca\(^{2+}\), our SAXS-based models detect an early opening of the gelsolin structure. Compared with our EGTA/gelsolin model, the 0.5 \(\text{nm}\) free Ca\(^{2+}\) gelsolin model exhibited a higher \(\chi\) fit value (1.3 versus 0.9) for the EGTA/gelsolin crystal structure. Thus, it appears that there are early global structural changes at these concentrations that are not detected as changes in surface-exposed residues, but are reflected in a loosening of the structure.

Insight into the global structural changes occurring during the sharp transition from Stage 1 to Stage 2 of the Ca\(^{2+}\) activation of gelsolin proposed by Kisela\textit{r et al.} (19) can be understood by studying the structure solved at pCa 7.4 (Fig. 4 and Table 2). This model suggests that the predominant shape of gelsolin involves apparent unwinding from both ends. A model of the Stage 2 structure is that for pCa 5.7 (Fig. 4 and Table 2). The model that best fit these data hints that both N- and C-terminal halves have opened up, but that the contact points across G3 and G4/G5 (presumably via the g3–g4 linker) are not yet completely lost. Nonetheless, it appears that the actin-binding domains G2 and G5 are exposed at this stage of the Ca\(^{2+}\) activation process. If intracellular [Ca\(^{2+}\)]\(_{\text{free}}\) is near this Stage 2 activation level (\(\sim 10\text{ \text{μM}}\)), then it is this observed structural model that would be competent in binding to F-actin.

Models of the data at pCa 4.3 and 4 (Fig. 4 and Table 2) represent a population shift in the global structure of gelsolin from Stage 2 to Stage 3 of the calcium activation process proposed by Kisela\textit{r et al.} (19). It is at this stage where the six individual domains become more distinct in our models, and we can envision that addition of further Ca\(^{2+}\) causes a concerted opening of the G3/G4 interdomain contacts at the \(\sim 70\)-amino acid residue g3–g4 linker unwinds. Comparing relative
Using the crystal structures of the two halves of gelsolin as they fit within our Ca$^{2+}$-activated gelsolin model, we positioned our Ca$^{2+}$/gelsolin model onto the Holmes F-actin model (Fig. 7). The SAXS data provide a constraint for the spatial dimensions of the g3–g4 linker, which in this overlay is clearly sufficient for positioning of the two gelsolin halves onto the F-actin model. One can conclude that the dimension of the g3–g4 linker within Ca$^{2+}$-activated gelsolin is compatible with the dimension of the F-actin cross-sectional distance, and no further opening of the structure would be required for binding. Fig. 7 also highlights the additional conformational step that would be required of the g1–g2 linker upon binding actin. This linker region, also known as the WH2 (WASP homology 2) domain, contains a consensus sequence (FKHVXPN) found in many actin regulatory proteins. Amazingly, simply by mutating its short g1–g2 linker to include the entire WH2 sequence from gelsolin, CapG is converted from a capping-only protein to one capable of severing actin as well (16). Crystallographic and modeling analyses of this CapG “gain-of-function” mutant led to the conclusion that the exact length of this g1–g2 linker segment is critical for actin filament severing yet plays little role in actin filament capping or actin monomer binding functions (16). Our SAXS-based model of fully activated gelsolin overlaid with the crystal structure of Ca$^{2+}$-activated N-terminal gelsolin bound to actin clearly demonstrates the additional conformational step that would be required of the g1–g2 linker sequence to properly position the G1-severing domain, thus advancing our current understanding of the severing mechanism of F-actin by gelsolin.

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