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FOR MORE INFORMATION

Prof. Mark R. Chance, Department of Physiology & Biophysics, Albert Einstein College of Medicine
mrc@aecom.yu.edu

Visualizing the Ca²⁺ Dependent Activation of Gelsolin using Synchrotron Footprinting

Janna G. Kiselar^{1,3}, Paul A. Janmey⁴, Steven C. Almo^{2,3} and Mark R. Chance^{1-3*}

¹Department of Physiology & Biophysics and ²Biochemistry, and ³Center for Synchrotron Biosciences, Albert Einstein College of Medicine, Bronx, NY; ⁴Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA

Gelsolin is a Ca²⁺-dependent protein composed of six homologous subdomains (denoted S1-S6) that severs actin filaments (F-actin) and caps the fast-growing barbed end with high affinity. The helical tail of S6, termed the "latch" interacts in a non-covalent manner with the F-actin binding helix of S2 making its actin-binding sites inaccessible in the absence of Ca²⁺. Upon Ca²⁺ activation protein undergoes substantial changes in the structure, involving a number of rearrangements that reveal actin filaments and actin monomer binding sites. Synchrotron protein footprinting was used to reveal detailed structural changes that occur in the Ca²⁺ dependent activation of gelsolin.

Only modest structural information is available regarding the Ca²⁺-induced reorganization accompanying gelsolin activation. Specifically a structure of S4-S6 bound to Ca²⁺ and actin at 3.4 Å resolution shows details of specific rearrangements involving S4 and S6 (**Figure 1d**), which are thought to represent steps in the Ca²⁺ activation process. The most dramatic is the disruption of the extended β-sheet between S4 and S6 with S6 rotating away from S4 and moving adjacent to S5; however, it is unclear which of the above Ca²⁺-binding processes induces this reorganization. There exists a need to develop experimental approaches that directly link Ca²⁺-binding events with structural rearrangements at the atomic scale.

Synchrotron protein footprinting provides a quantitative approach to monitor changes in surface accessibility of specific amino acid side chains. Hydroxyl radicals generated from millisecond exposure of aqueous solutions to unattenuated "white" synchrotron radiation result in the stable oxidative modification of solvent accessible amino acid side chains. The specific extents and sites of oxidative modification are quantified by proteolytic digestion and mass spectrometry

[Kiselar, J.G., et al., *Int J Radiat Biol.*, 78, 101-14, (2002); Guan, J.Q., et al., *Biochemistry*, 41, 5765-75, (2002)]. The most reactive residues, which represent the experimental probes for this method, include surface accessible cysteine, methionine, phenylalanine, tyrosine, tryptophan, histidine, proline, and leucine side chains.

More than 80 peptides segments within the structure, covering 95% of the sequence in the molecule, were examined by this approach for their solvent accessibility as a function of Ca²⁺ concentration in solution. Twenty-two of the peptides exhibited detectable oxidation; for seven, the oxidation extent was

seen to be Ca²⁺ sensitive. Ca²⁺ titration isotherms (**Figure 2a-d**) monitoring the oxidation within residues 49-72 (within S1), 121-135 (S1), 162-166 (S2) and 722-748 (S6) indicate a three-state activation process with the intermediate that was populated at a Ca²⁺ concentration of 1-5 μM that is competent for capping and severing activity. A second structural transition with a mid-point of ~60-100 μM (data shown in **Figure 2a-d**), where the accessibility of the above four peptides is further increased, is also observed.

Tandem mass spectrometry data showed that buried residues within the helical "latch" of S6 (including Pro745) that contact an F-actin binding site on S2 and buried F-actin binding residues within S2 (including Phe163) become exposed upon Ca²⁺ binding (**Figure 1b-c**), suggesting that the helical "latch" is released in the first transition coincident with the occupancy of a high-affinity Ca²⁺-binding site in S6. However, residues within S4 that are part of an extended β-sheet with S6 (including Tyr453 that is shown in **Figure 2c**) are revealed only in the subsequent transition at higher Ca²⁺ concentrations (**Figure 2e**); the disruption of this extended contact be-



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tween S4 and S6 (and likely the analogous contact between S1 and S3) likely results in an extended structure permitting additional functions consistent with the fully activated gelsolin molecule. The location of eight amino acids that were oxidized in the presence of Ca^{2+} only are illustrated in **Figure 1a-c**.

The results provide clear evidence for a three state, Ca^{2+} -induced activation process. State 1 corresponds to the " Ca^{2+} -free" form, state 2 is the intermediate observed in these studies that involves some unlatching of the structure, and state 3 is the Ca^{2+} saturated, fully activated form. The transition between states 1 and 2

occurs at sub-micromolar concentrations, which is in a good agreement with the previous biochemical studies and is accompanied by the binding of multiple Ca^{2+} ions. The transition between states 2 and 3 is mediated by occupancy of lower affinity binding sites and accompanied by the binding of 2-3 additional Ca^{2+} ions.

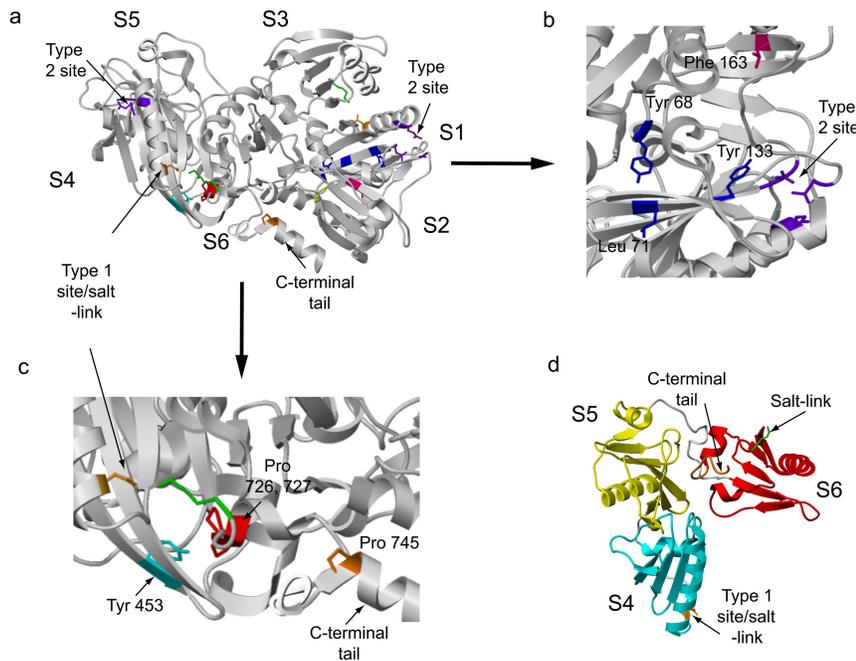


Figure 1. (a) The subunit structure of gelsolin. The buried residues that are revealed in the presence of Ca^{2+} are shown. The type 2 Ca^{2+} binding sites in S1 and S4 are colored in purple. The type 1 Ca^{2+} binding site in S4 and S6 are colored in gold and green, respectively. The residues from S4 and S6 form a salt-link between the sub-units. (b) A close-up of the S1 type 2 site with the adjacent reactive residues labeled. (c) A close-up of the S4 type 1 site with the reactive residues labeled. (d) The structure of the activated C-terminal half of gelsolin.

Figure 2. Ca^{2+} titration isotherms indicating changes in oxidation extent for specific peptides after exposure of gelsolin to the x-ray beam for 80 milliseconds. (a-e) The % oxidized peptide is shown on the right-hand side of the y-axis; on the left-hand side, the transition extent is indicated. The solid lines represent the fitting of the curves as described in the text. (f) oxidized peptides within gelsolin show no changes in oxidation upon titration by Ca^{2+} . The % oxidized peptide is shown on the right- and left-hand sides.

