Mechanism of F-actin crosslinking by filamin A and the anti-inflammatory functions of plasma gelsolin in bodily fluids

Akademisk avhandling

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av

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I. **Modifications of cellular responses to lysophosphatidic acid and platelet-activating factor by plasma gelsolin.**
Teresia M. Osborn, Claes Dahlgren, John H. Hartwig, Thomas P. Stossel
Am J Physiol Cell Physiol 292:1323-1330, 2007.

II. **Decreased plasma gelsolin levels in rheumatoid arthritis.**
Teresia M. Osborn, Margareta Verdrengh, Thomas P. Stossel, Andrej Tarkowski, Maria Bokarewa
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III. **Structural basis of filamin A functions.**
Fumihiko Nakamura*, Teresia M. Osborn*, Christopher A. Hartemink, John H. Hartwig, Thomas P. Stossel
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Mechanism of F-actin crosslinking by filamin A and the anti-inflammatory functions of plasma gelsolin in bodily fluids

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Abstract

Gelsolin (GSN) and filamin A (FLNa) are two actin-binding proteins discovered in our laboratory over 30 years ago. GSN is a calcium-activated actin severing and barbed end capping protein that is expressed as both intracellular and extracellular (plasma gelsolin, pGSN) isoforms. pGSN is present at relatively high concentrations (~ 200 μg/ml) in blood, but its extracellular functions have not been determined. pGSN levels decrease during acute inflammation and low levels correlate negatively with survival. Re-administration of pGSN to severely injured animals can rescue them from death, although the mechanism for this is unknown. pGSN levels during chronic inflammation have not been reported. FLNa is an important architectural component of three-dimensional actin networks in cells. It is an elongated homo-dimer that efficiently crosslinks F-actin into a gel in contrast to the gel-solating properties of GSN. Each subunit has an N-terminal "actin-binding domain" (ABD) followed by two rod-like domains and a C-terminal self-association domain. FLNa mediates actin-membrane connections, serves as a scaffold for >50 different binding partners, and FLNa-F-actin crosslinks accommodate cell shape changes and motility. However, as of yet there have not been sufficient details concerning FLNa's structure to fully explain its multiplicity of functions.

pGSN has lipid-binding sites and has been shown to bind to lysophosphatidic acid (LPA), a potent cell-activating phospholipid. Based on this, a new hypothesis positing pGSN as an anti-inflammatory protein was formed. Using platelets and neutrophils isolated from human blood, the effects of recombinant pGSN on platelet P-selectin exposure and neutrophil oxygen radical production induced by LPA and another structurally related phospholipid, platelet-activating factor (PAF), were investigated. Results showed that pGSN modulated cellular activation induced by both of these inflammatory phospholipids. In order to investigate pGSN levels during chronic inflammation, plasma and synovial fluids from patients with rheumatoid arthritis were analyzed. pGSN levels were lower in plasma from patients than age and gender matched healthy controls, and further reduced in synovial fluid.

To examine the mechanism behind FLNa's potency as a F-actin crosslinker, the FLNa-F-actin interaction was investigated by binding and gel-point assays, electron microscopy, and real-time video microscopy using full-length and truncated FLNa molecules. A new F-actin binding site was identified, which functions in conjunction with dimerization, long flexible subunits, and the previously identified ABD, to explain high avidity binding to F-actin. The results also show that crosslinks are rigid structures and that the self-association domains determine high angle branching. The C-T domain of FLNa, which binds many partners, has a compact structure compared to the elongated N-T two-thirds of the protein, does not associate with F-actin and can bind partners while FLNa is bound to F-actin.

In conclusion, these findings demonstrate a novel function of pGSN as a modulator of phospholipids, a finding that may be important for inflammation, and that pGSN levels are decreased during chronic inflammation in addition to previously documented acute conditions. The mechanism of FLNa crosslinking of F-actin can be explained by the intrinsic structure and properties of the FLNa molecule.

Keywords: cytoskeleton, crosslinking, F-actin, filamin, gelsolin, inflammation, plasma, platelet-activating factor, lysophosphatidic acid, rheumatoid arthritis.

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