

Stossel/Cunningham

Gelsolin-Polyphosphoinositide Interaction

FULL EXPRESSION OF GELSOLIN-INHIBITING FUNCTION BY POLYPHOSPHOINOSITIDES IN VESICULAR FORM AND INACTIVATION BY DILUTION, AGGREGATION, OR MASKING OF THE INOSITOL HEAD GROUP*

(Received for publication, August 15, 1988)

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Calcium activates, and the polyphosphoinositides phosphatidylinositol 4-monophosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP₂) inhibit the mechanical severing of actin filaments by gelsolin. Previous work indicated that the physical state of the two phospholipids is important for their effects in this system. This study correlates tests of gelsolin's severing function with quasiclastic light scattering measurements of the size of mixed lipid particles and shows that the previously demonstrated diminution of the maximal effect of PIP₂ in micellar form by aggregation of the micelles or mixing with other phospholipids is not the result of an absolute requirement for small lipid particles, but rather the masking of critical sites by aggregation, by sequestration in multilamellar vesicles, or by dilution of the polyphosphoinositides below a critical concentration. Large unilamellar vesicles of PIP and, importantly, PIP₂ at low molar ratios (<3%) in mixed lipid vesicles of composition similar to plasma membranes are as active as PIP₂ micelles. Aggregation or masking of polyphosphoinositide head groups by neomycin or profilin, respectively, blocked inhibition of gelsolin. Experiments with bilayer-forming phospholipids or with Triton X-100 indicate that a critical number of PIP₂ molecules may be required for incipient effects on a gelsolin molecule. The actin and polyphosphoinositide binding protein profilin competed with gelsolin for binding PIP₂ with a stoichiometry also suggesting binding to multiple PIP₂ molecules. The membrane constituents sphingosine and cholesterol blocked the effect of PIP₂ on gelsolin when added alone, but did not affect PIP₂ when incorporated into mixed lipid bilayers containing phosphatidylinositol. The results suggest that profilin, small changes in membrane lipid composition, and, especially, membrane PIP₂ concentration could have large effects on the modulation of gelsolin function *in vivo*.

Actin polymerization accompanies receptor-mediated responses in many cell types. One candidate to link cell activation to changes in actin polymerization is the protein gelsolin which severs actin filaments, binds to their fast-exchanging ends, and produces nucleation sites for new actin filaments which could assemble from the large pool of unpo-

lymerized actin in quiescent cells (reviewed by Stossel *et al.*, 1985; Harris, 1987; Yin, 1987; Matsudaira and Janmey, 1988). Ca²⁺ activates gelsolin's actin-severing and -nucleating functions (Yin and Stossel, 1979), while the polyphosphoinositides (PPIs)¹ phosphatidylinositol 4-phosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP₂) inhibit actin-severing by gelsolin and free gelsolin-blocked filament ends for assembly (Janmey *et al.*, 1987). Gelsolin's effects may therefore be directly coupled to changes in the cytoplasmic concentrations of calcium ions and phospholipid metabolites that occur as a result of cell activation. The recent discovery that gelsolin associates with the plasma membranes of macrophages and platelets, and that agents causing phosphoinositide turnover perturb this association (Hartwig *et al.*, 1988) suggest that gelsolin may regulate actin assembly at the cell periphery following stimulation.

Previous studies showed that PIP and PIP₂ inhibited gelsolin function *in vitro* maximally when in the form of micelles (Janmey *et al.*, 1987). PIP₂ spontaneously forms small, 80 nm diameter micelles in aqueous suspension, whereas PIP requires extensive sonication to form equivalent structures (Hendrickson, 1969; Sugiura, 1981). Aggregation of PIP₂ micelles by millimolar concentrations of Mg²⁺, nonionic detergents, or incorporation of PIP₂ in an excess of multilamellar phosphatidylcholine vesicles decreased the ability of PIP₂ to inhibit the actin filament-severing function of gelsolin, although about a third of maximal activity persisted in a large excess of this vesicle-forming phospholipid (Janmey *et al.*, 1987). These findings underscored the importance of the physical state of PPIs for their effects on gelsolin and suggested that particle size might be an important determinant of PPI interaction with gelsolin. The suggestion requires an answer since PPIs presumably reside in planar cell membranes and not as small micelles *in vivo*. This paper addresses this issue and examines other agents that interact with PPIs, including studies of their effects on lipid particle size. In particular, it documents that PPIs have functional effects when entirely in the vesicular form likely to exist in living cells.

EXPERIMENTAL PROCEDURES

Proteins—Human plasma gelsolin and rabbit skeletal muscle actin were prepared as described in previously studies (Chaponnier *et al.*, 1986), and a portion of the actin was labeled with pyrene iodacetate.

* This work is supported by United States Public Health Service Grants AR38910, HL19429, and by the Council for Tobacco Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ The abbreviations used are: PPI, polyphosphoinositide; PIP, phosphatidylinositol 4-monophosphate; PIP₂, phosphatidylinositol 4,5-bisphosphate; PC, phosphatidylcholine; PI, phosphatidyl inositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; LSS, laser light scattering; EGTA, [ethylenebis(oxyethylene)]triacetic acid.