Gelsolin Binding to Phosphatidylinositol 4,5-Bisphosphate Is Modulated by Calcium and pH*

(Received for publication, April 24, 1997, and in revised form, May 22, 1997)

Keng-Mean Lin‡, Elizabeth Wenegieme‡, Pei-Jung Lu§, Ching-Shih Chen§, and Helen L. Yin‡§

From the ‡Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas and the §Division of Medicinal Chemistry and Pharmaceutical, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536-0082

The actin cytoskeleton of nonmuscle cells undergoes extensive remodeling during agonist stimulation. Lamellipodial extension is initiated by uncapping of actin nuclei at the cortical cytoplasm to allow filament elongation. Many actin filament capping proteins are regulated by phosphatidylinositol 4,5-bisphosphate (PIP2), which is hydrolyzed by phospholipase C. It is hypothesized that PIP2 dissociates capping proteins from filament ends to promote actin assembly. However, since actin polymerization often occurs at a time when PIP2 concentration is decreased rather than increased, capping protein interactions with PIP2 may not be regulated selectively by the bulk PIP2 concentration. We present evidence that PIP2 binding to the gelsolin family of capping proteins is enhanced by Ca2+. Binding of PIP2 to gelsolin was increased approximately 8-fold and 4-fold, respectively, by 10 μM Ca2+ and by Ca2+ requirement was reduced by lowering the pH from 7.5 to 7.0. Studies with the NH2- and COOH-terminal halves of gelsolin showed that PIP2 binding occurred primarily at the NH2-terminal half and Ca2+ exposed its PIP2 binding sites through a change in the COOH-terminal half. Mild acidification promotes PIP2 binding by directly affecting the NH2-terminal half sites. Our findings can explain increased PIP2-induced uncapping even as the PIP2 concentration drops during cell activation. The change in gelsolin family PIP2 binding affinity during cell activation can impact divergent PIP2-dependent processes by altering PIP2 availability. Cross-talk between these proteins provides a multilayered mechanism for positive and negative modulation of signal transduction from the plasma membrane to the cytoskeleton.

Phosphoinositides are important in signal transduction, both as precursors to signaling molecules and as physical anchors and regulators of proteins (1, 2). Among these, the D4 phosphoinositide, phosphatidylinositol 4,5-bisphosphate (PIP2), has been implicated as a potential mediator of actin cytoskeletal rearrangements (3, 4). PIP2 modulates many actin regulatory proteins. These include the following: actin severing and/or capping proteins (gelsolin (5), CapG (6), and capping protein (also known as Cap Z (7)), monomer-binding proteins (profilin (8) and coflin (9)), and other actin-binding proteins (α-actinin (10) and vinculin (11)). It has been hypothesized that PIP2 induces explosive actin assembly by dissociating capping proteins from filament ends and releasing actin monomers from actin-sequestering proteins (3, 7, 12). The involvement of PIP2 in actin polymerization is supported by recent experiments that show that Rac1 and RhoA, monomeric GTPases of the Rho family that have well defined effects on the cytoskeleton (13), stimulate the synthesis of PIP2 (14–16). Furthermore, manipulations that alter the availability of PIP2 in cells have profound effects on agonist and/or Rac1-induced filament elongation, actin polymerization, and cell motility (16, 17).

Agonist-stimulated cells exhibit complex Ca2+ oscillations and pH transients. These signals alter the binding of gelsolin and CapG to actin, by inducing a conformational change (6, 25–27). In this study, we tested the effect of Ca2+ and pH on the binding of the gelsolin family proteins to PIP2 and found that they affect PIP2 binding in an interdependent manner. We identified the domains in gelsolin that impart such regulation and elucidated the relation between the NH2-terminal and COOH-terminal halves of the protein. Since gelsolin modulates the activity of many PIP2-regulated proteins with important signaling functions in vivo (28) and in vitro (29–31), our results have important implications for how the gelsolin family proteins are regulated during agonist signaling and how the activity of other PIP2-dependent cytoskeletal and noncytoskeletal proteins can be coordinated.

EXPERIMENTAL PROCEDURES

Expression and Purification of Recombinant CapG, Gelsolin, and Gelsolin Domains—Gelsolin has six semihomologous domains (S1–6), which can be further divided into two functional halves (32). The expression vectors for the gelsolin NH2-terminal half (S1–3), gelsolin S1, gelsolin S2–3, and CapG have been described previously (33–35). The full-length gelsolin expression vector (encompassing the entire human plasma gelsolin coding sequence) was constructed by ligating gelsolin cDNA to pet3a via the BamHI site. Recombinant proteins were expressed in bacteria and purified using sequential anion and cation.
exchange chromatography (34). Protein concentration was determined by the method of Bradford (36), and protein purity was assessed by SDS-polyacrylamide gel electrophoresis.

The COOH-terminal half expression vector was constructed by using polymerase chain reaction to generate a fragment encompassing human plasma gelsolin nucleotides 1288–1753. The forward primer contains a XhoI site (ACC TCC ACT CTG GCC GGC), and the reverse primer has a SmaI site (CAA CAG CCC GGG TGG CT). The polymerase chain reaction product was cloned into Bluescript KS+ via the XhoI/SmaI sites. This construct was digested with SmaI and blunt end-ligated with a downstream gelsolin fragment. The fragment was excised with BamHI from full-length gelsolin cDNA in Bluescript KS+ (gelsolin SmaI site at nucleotide 1750 and vector multiple cloning SmaI site downstream of the termination codon). The resultant cDNA was digested with SpeI (in the 3′ multiple cloning region, downstream of SmaI) and filled in with CT nucleotides to create a site with a two-base overhang compatible with that of HindIII. The other end was released by digestion with XhoI and ligated to PGEX KS vector that was linearized with HindIII (site partially filled in with nucleotides AG to generate a two-base overhang compatible with the partially filled in SpeI) and XhoI. The fusion protein contained a 30-kDa GST followed by a 40-kDa gelsolin COOH-terminal half. The COOH-terminal gelsolin was cleaved from GST bound to a column with thrombin.

Phospholipid—PIP$_2$ was purchased from Calbiochem. Micelles were prepared by dissolving the dried lipid in water to a final concentration of 2 mg/ml and sonicating for 5 min. at maximum power (model W185, Heat Systems Ultrasonics, Inc., Farmingdale, NY). Large unilamellar vesicles at a 5:1 phosphatidylcholine:PIP$_2$ ratio were made with an Ultraturrax homogenizer (Janke and Kunkel, Germany) in 25 mM Hepes, 100 mM KCl, 0.4 mM EGTA, 0.5 mM β-mercaptoethanol, pH 7.5, with or without 0.4 mM NaN$_3$, 0.25 mM CaCl$_2$, 0.5 mM MgCl$_2$, pH 7.5, with or without 0.5 mM MgCl$_2$. 20444 nm. The amount of unbound protein was determined from the trough in the absorbance peak. The decrease in fluorescence emission at 320 nm was plotted as a function of PIP$_2$ concentration, and the fluorescence change was determined by the equation,

\[ \Delta F = \frac{\Delta F_{\text{max}} \times [\text{lipid}]]}{K_d + [\text{lipid}]]} \]  
(Eq. 3)

where $\Delta F$ is the fluorescence quenching at a given PIP$_2$ concentration, $\Delta F_{\text{max}}$ is the total fluorescence quenching of the protein saturated with ligand, and [lipid] is the concentration of PIP$_2$. $K_d$ was calculated using the equation,

\[ K_d = \frac{[\text{protein}][\text{lipid}]}{[\text{protein-lipid}]} \]  
(Eq. 1)

Equilibrium Gel Filtration—The method of Hummel and Dreyer (39), as modified by Machesky et al. (37) was used. A Superose 12 HR 10/30 column (Pharmacia) was equilibrated with CapG (ligand) in a buffer containing 25 mM Hepes, 75 mM KCl, 0.5 mM dithiobiotreitol, 1.8 mM NaN$_3$, 0.05 mM CaCl$_2$, pH 7.5, at room temperature. 100 μl of the equilibration buffer containing CapG was incubated with PIP$_2$ micelles for 30 min and loaded onto the column. The column was developed with the equilibration buffer containing CapG at 0.25 ml/min, and 0.3-ml fractions were collected. CapG concentration in the column fractions was monitored by UV absorption. The amount of CapG bound to PIP$_2$ was determined from the trough in the absorbance peak. Multiple runs using equilibration CapG concentrations of 0.96, 1.3, 2.6, and 3.9 μM and PIP$_2$ concentrations of 34, 46, 68, and 91 μM were done. $K_d$ was determined by the equation,

\[ r = \frac{B_{\text{max}}[\text{protein}][\text{lipid}]}{K_d + [\text{protein}][\text{lipid}]} \]  
(Eq. 2)

where $r$ is the ratio of protein bound to each PIP$_2$ molecule at a given PIP$_2$ concentration and $B_{\text{max}}$ is the maximum number of protein bound per PIP$_2$ at saturation.

Quenching of Intrinsic Tryptophan Fluorescence—Fluorescence spectra were recorded at 30 °C with a QM-1 fluorometer (Photon Technology International, Canada). 2 ml of a protein solution (0.3 μM, 30 °C) in 25 mM Hepes, 100 mM KCl, 0.4 mM EGTA, 0.5 mM β-mercaptoethanol, pH 7.5, with or without 36 μM free Ca$^{2+}$ were placed in a 1-cm square quartz cuvette and stirred with a minimmagnetic stirrer. After allowing 5 min for equilibration, the tryptophan fluorescence spectrum was recorded by excitation at 292 nm. The excitation and emission beam slits were set at 3 and 2 nm bandwidth, respectively. PIP$_2$ micelles (at final PIP$_2$ concentrations ranging from 0.042 to 32.3 μM, depending on the protein studied) were added at 2-μl increments, and the fluorescence spectra were recorded 5 min after each addition. The total volume of micelles added did not exceed 2% of the initial protein solution volume. The decrease in fluorescence emission at 320 nm was plotted as a function of PIP$_2$ concentration, and the fluorescence change was assumed to be proportional to the concentration of the protein-phosphoinositide complex. Data were analyzed as described by Ward (40). The apparent dissociation constant, $K_d$, was calculated using the equation,
**Gelsolin and CapG Interactions with PIP₂**

**TABLE I**

**Binding of CapG to PIP₂**

\[ K_d \] for fluorescence titration was calculated using Equation 4. \[ K_d \] values for gel filtration data were calculated with Equation 1, assuming a stoichiometry of 2. Values shown are mean ± S.E., determined at pH 7.5.

| Fluorescence | Gel filtration |
|--------------|----------------|
| EGTA | Ca²⁺ | Gel | EGTA | Ca²⁺ | Equilibrium (Ca²⁺) |
| 24.4 ± 5.9 (n = 3) | 6.0 ± 0.8 (n = 3) | 1.7 | 1.1 ± 0.03 (n = 3) | 69.0 ± 5.3 (n = 5) | 29.4 ± 2.5 (n = 7) | 7.0 ± 0.5 (n = 5) |

\( ^a \) p, mol of PIP₂/mol of CapG, average of two determinations.

\( ^b \) h, Hill coefficient.

\( ^c \) n, number of independent experiments.

SigmaPlot. Alternatively, the intrinsic association constant (\( K_a \)) as well as the stoichiometry of binding (p) can be derived using the graphical method of Stinson and Holbrook (41),

\[
\frac{1}{(1 - \theta)K_a} = \frac{[lipid]}{\theta} - \frac{[protein]}{\theta}
\]

(Eq. 4)

where \( \theta \) is the fractional binding (\( \Delta F/\Delta F_{max} \)), \( p \) is the stoichiometry of binding, [lipid] \( _p \) is the total concentration of PIP₂, and [protein] \( _p \) is the total acceptor concentration. When \( 1/(1 - \theta) \) is plotted against [lipid]/\( \theta \), a straight line with a slope of \( K_a \) and an intercept of [protein]/\( \theta \) is obtained. The stoichiometry of interaction (p) can be calculated by dividing the intercept with the protein concentration.

**Measurement of Free Ca²⁺ Concentration and pH**—The concentrations of free Ca²⁺ in EGTA containing solutions with varying amounts of Ca²⁺ were measured with Ca²⁺-sensitive dyes. 5 μM Fura-2 was used to determine Ca²⁺ concentrations below 1 μM. Free Ca²⁺ concentration was calculated (26) assuming the \( K_d \) of the Fura-2-Ca²⁺ complex is 229 nM at pH 7.0 and 144 nM at pH 7.5. Calcium green 5N (Molecular Probes, Eugene, OR) was used to measure Ca²⁺ concentrations higher than 1 μM, and free Ca²⁺ concentration was calculated assuming a \( K_d \) of 14 μM.

**RESULTS**

**CapG Binding to PIP₂**—Small zone gel filtration analyses showed that CapG bound to PIP₂ micelles in a dose-dependent manner. Micelle-bound CapG eluted in the void volume that was well separated from the free protein peak (Fig. 1). Binding to phosphatidylcholine-PIP₂ vesicles gave similar results (data not shown), suggesting that micelles could be used to assess binding, although it is not a physiological substrate. To facilitate comparison under different binding conditions and between different proteins, we attempted to calculate a \( K_d \). Equilibrium binding studies suggest that each CapG binds two PIP₂ molecules (see below). Assuming this stoichiometry, the apparent \( K_d \) for binding to PIP₂ micelles (calculated using Equation 1) was 69.0 μM in 1 mM EGTA, and 29.4 μM in the presence of 36 μM Ca²⁺ at pH 7.5 (Table I). These values represent the upper limit, since measurements were not made under equilibrium conditions.

To determine if there is indeed a Ca²⁺-induced change, equilibrium binding studies based on the quenching of CapG intrinsic tryptophan fluorescence by PIP₂ were performed. This method has been used to study the binding of profilin (40), phospholipase Cδ (44), and dynamin pleckstrin homology domain (45) to PIP₂. CapG had an emission maximum of 327 nm, and 36 μM Ca²⁺ produced a small reduction in fluorescence intensity (the ratio of peak fluorescence in EGTA/Ca²⁺ is 0.92 ± 0.05 (mean ± S.E., n = 5) (Fig. 2A and B)). PIP₂ induced a dose-dependent and saturable decrease in intrinsic fluorescence, without shifting the emission maximum. Micelles alone without CapG did not have significant emission (data not shown). A plot of CapG fluorescence quenching versus PIP₂ concentration showed that saturation was reached at a lower PIP₂ concentration in the presence of Ca²⁺ than in EGTA (Fig. 3A). The \( K_d \) values for binding at pH 7.5, calculated according to Equation 3, were 31.9 and 8.4 μM in EGTA and Ca²⁺,
Gelsolin and CapG Interactions with PIP₂

Fig. 3. Analysis of CapG fluorescence titration data. A, binding curves plotting \( F_{\text{max}} - F \) versus \( \text{PIP}_2 \) concentration. Closed and open circles represent experimental points in 0.4 mM EGTA or 36 \( \mu \text{M} \) Ca²⁺. Dotted lines are fitted to the experimental points, and \( F_{\text{max}} \) values were obtained. \( K_d \) values with and without Ca²⁺ were 8.4 and 31.9 \( \mu \text{M} \), respectively, for this experiment. B, Hill plot of the titration data. The Hill equation is rearranged and plotted to show the relation between the log(\( Y/(1-Y) \)) and log(\( \text{lipid}_{\text{max}} \)), where \( Y \) is the fractional saturation. \( h \), the Hill coefficient, is derived from the slope (1.1 and 0.97 in the presence of Ca²⁺ and EGTA for this experiment).

Useful for comparison among different proteins.

Equilibrium gel filtration validated the \( K_d \) derived by fluorescence titration. The column was preequilibrated with CapG, and \( \text{PIP}_2 \) incubated with CapG in the equilibrating buffer was added. The column was then developed with CapG containing equilibration buffer. CapG bound to \( \text{PIP}_2 \) migrated faster, in-depending on the amount in the trailing fractions (trough) (Fig. 4). However, in EGTA, gelsolin has a much higher \( K_d \) than CapG, suggesting that Ca²⁺ decreases the \( K_d \) by half at pH 7.0, while 4.5 \( \mu \text{M} \) Ca²⁺ was required to produce the same effect at pH 7.5. Both Ca²⁺ concentrations are well within the range achieved following agonist stimulation, particularly at the cytoplasm immediately subjacent to the plasma membrane.

\( Ca²⁺ \) and pH Regulation of Gelsolin Domains—To determine which part of gelsolin contributes to the Ca²⁺ and/or pH dependence of \( \text{PIP}_2 \) binding, we examined the \( \text{PIP}_2 \)-binding characteristics of several gelsolin domains. Gelsolin contains six segmental repeats, S1–6 (32). The NH₂-terminal half encompassing S1–3 binds actin independently of Ca²⁺ (48) and has two known \( \text{PIP}_2 \) binding sites and potentially a third un-mapped site (35, 49, 50). The COOH-terminal half (S4–6), which requires Ca²⁺ to bind actin (51), has not been examined previously for \( \text{PIP}_2 \) binding.

Unlike full-length gelsolin, the gelsolin NH₂-terminal half behaved well during fluorescence titration (Fig. 6A). It bound \( \text{PIP}_2 \) with high affinity, and saturation was reached at a slightly lower \( \text{PIP}_2 \) concentration in EGTA than in Ca²⁺ (the opposite of full-length gelsolin and CapG). The \( K_d \) values for the experiment shown in Fig. 6A were 1.2 and 2.9 \( \mu \text{M} \), respectively. The stoichiometry of binding derived from Fig. 6B was 3.4. This value is twice that of CapG, confirming that gelsolin NH₂-terminal half has more \( \text{PIP}_2 \) binding sites (33). Gel filtration studies confirmed that Ca²⁺ increased the \( K_d \). The Hill coefficient of 1.1 ± 0.03 (Fig. 6C, Table II) suggested that binding was noncooperative and that the sites bound \( \text{PIP}_2 \) independently. S1, which has one \( \text{PIP}_2 \) site, bound 1.6 mol of
PIP$_2$ with a $K_d$ of 4.2 μM in EGTA, while S2–3 bound 2.1 μmol of PIP$_2$ with a $K_d$ of 1.0 and 2.9 μM in EGTA and Ca$^{2+}$, respectively (Table II).

The gelsolin COOH-terminal half bound PIP$_2$ with much lower affinity (approximately 7-fold higher $K_d$ by fluorescence measurements) than the NH$_2$-terminal half (Table II). It is therefore probably not involved in PIP$_2$ binding per se. As with the NH$_2$-terminal half, binding to the COOH-terminal half was reduced in Ca$^{2+}$ (Fig. 7C). This is in sharp contrast to the large Ca$^{2+}$-enhancement of PIP$_2$ binding to full-length gelsolin. The

**FIG. 5. Effects of Ca$^{2+}$ and pH on the binding of gelsolin to PIP$_2$, as determined by small zone gel filtration.** 2.3 μM gelsolin was incubated with 56.7 μM PIP$_2$ in a pH 7.5 or 7.0 buffer containing 0.4 μM EGTA and increasing amounts of CaCl$_2$. The free Ca$^{2+}$ concentration was determined as described under “Experimental Procedures.” A–F, gelsolin elution profiles. A–C, pH 7.5; D–F, pH 7.0. The $K_d$ values at pH 7.5, from left to right, are 323.2, 298.6, and 118.6 μM, respectively. $K_d$ values at pH 7.0, from left to right, are 345.2, 119.7, and 16.2 μM, respectively. G, plot of $K_d$ versus Ca$^{2+}$ concentration, at two different pH values.

**TABLE II**

| Protein          | $K_d$ (μM) | Fluorescence$^a$ | Gel filtration |
|------------------|------------|------------------|----------------|
|                  | EGTA       | Ca$^{2+}$        | $p^b$ | $h^c$ | EGTA       | Ca$^{2+}$        |
| Gelsolin (S1–6)  | 3.0        | 20.2             | 6 (3.0) | 305.4 ± 20.2 (n = 7)$^d$ |
| NH$_2$-half (S1–3)| 1.3 ± 0.3  | 2.9 ± 0.7        | 3.4 | 1.0 ± 0.03 |
| S1               | 4.2 ± 2.3  | 1.6              | 2.1 | 0.9 ± 0.2 |
| S2–3             | 1.0 ± 0.2  | 2.9 ± 0.9        | 1.0 ± 0.1 |
| COOH-half (S4–6) | 9.7 ± 0.4  | 2.0 ± 1.2        | 0.9 ± 0.2 |

$^a$ Fluorescence titration results were mean ± S.E. for three independent experiments.

$^b$ $p$, stoichiometry of binding (mol of PIP$_2$/mol of protein), average of two determinations. Stoichiometry for gelsolin is assumed to be 3.

$^c$ $h$, Hill coefficient.

$^d$ $n$, number of gel filtration experiments.
opposite effects of Ca\(^{2+}\) on full-length and half-length gelsolins therefore cannot simply be due to nonspecific lipid aggregation. The pronounced enhancement of PIP\(_2\) binding to full-length gelsolin most likely reflects a Ca\(^{2+}\)-dependent exposure of the NH\(_2\)-terminal half PIP2 binding sites through a change in the COOH-terminal half. This conclusion is based on the observation that neither the NH\(_2\)-nor COOH-terminal halves are activated by Ca\(^{2+}\) to bind PIP\(_2\), and only the COOH-terminal half is known to undergo Ca\(^{2+}\)-induced conformational change (51).

Gelsolin NH\(_2\)-terminal half binding to PIP\(_2\) was enhanced by lowering pH. The \(K_d\) dropped from 8.2 to 3.4 \(\mu\)M between pH 7.5 and 7.0 in the presence of EGTA (Fig. 7A). In contrast, the gelsolin COOH-terminal half was not affected by pH (Fig. 7B).

**DISCUSSION**

Actin polymerization in response to agonist activation is frequently associated with a rise in cytosolic Ca\(^{2+}\), changes in PIP\(_2\) content, and intracellular pH. There is also compelling evidence that gelsolin, which severs and caps actin filaments in response to changes in Ca\(^{2+}\) and PIP\(_2\) concentration and pH, is involved in actin remodeling (17, 52–54). In this paper, we show that gelsolin and CapG binding to PIP\(_2\) is affected by physiologically relevant changes in Ca\(^{2+}\) and pH. The effects are not due to alterations in PIP\(_2\) structure per se but reflect changes in the proteins. This is the first report that PIP\(_2\) binding to any protein is directly modulated by signals generated during agonist stimulation and has implications for divergent PIP\(_2\)-dependent processes beyond a direct effect on the cytoskeleton.

The finding that gelsolin binding to PIP\(_2\) is promoted by Ca\(^{2+}\) is consistent with the current model for how gelsolin is activated by Ca\(^{2+}\) to bind actin (48, 51). Our deletion studies suggest that the extreme COOH terminus of gelsolin is critical to the inhibition of the NH\(_2\)-terminal actin binding sites, because gelsolin lacking the COOH-terminal 23 residues no longer requires Ca\(^{2+}\) to bind actin (56). We do not know at present whether actin binding and PIP\(_2\) binding are regulated identically. This question can now be addressed, because the actin and PIP\(_2\)-binding sites of gelsolin have been mapped (33, 50, 56–58) and the crystal structures of gelsolin S1 complexed with actin (57) and full-length gelsolin in EGTA\(^2\) have been solved recently.

Less is known about how pH affects gelsolin conformation. Selve and Wegner (59) first reported that pH 6 increases the rate of gelsolin binding to actin in the presence of Ca\(^{2+}\). Lamb et al. (26) subsequently showed that the Ca\(^{2+}\) requirement for gelsolin severing is reduced at pH 6.5 and abolished at pH below 6.0. pH 5 induces gelsolin unfolding, as determined by dynamic light scattering (26). We find that a less extreme pH drop potentiates Ca\(^{2+}\) activation of PIP\(_2\) binding to full-length

---

**Fig. 6. Effects of Ca\(^{2+}\) on the binding of the gelsolin NH\(_2\)-terminal half to PIP\(_2\), as determined by fluorescence titration.**

A, NH\(_2\)-terminal half (0.2 \(\mu\)M), with and without 36 \(\mu\)M Ca\(^{2+}\) in buffer containing 0.4 mM EGTA. PIP\(_2\) concentration ranged from 0.04 to 10.2 \(\mu\)M. Binding curves as described by Stinson and Holbrook (41). \(K_d\) is derived from the slope, and \(p\), the stoichiometry of binding, is derived from the intercept divided by the protein concentration. C, Hill plot of binding in the presence of EGTA.

**Fig. 7. Effects of pH on the binding of gelsolin NH\(_2\) and COOH-terminal halves to PIP\(_2\), as determined by gel filtration.**

A, gelsolin NH\(_2\)-terminal half (4 \(\mu\)M) binding to 11.3 and 22.7 \(\mu\)M PIP\(_2\) as a function of pH. Values shown are the mean of two determinations, and the range is indicated. B, COOH-terminal half (5.8 \(\mu\)M) binding to 22.7 \(\mu\)M PIP\(_2\) as a function of pH and Ca\(^{2+}\).

---

2 Burtnick, L. D., Koepf, E. K., Grimes, J., Jones, E. Y., Stuart, D. I., McLaughlin, P. J., and Robinson, R. C. (1997) Cell, in press.
Gelsolin and CapG Interactions with PIP₂

Gelsolin. Acidic pH increases the NH₂-terminal half binding to PIP₂ even without Ca²⁺ but has no effect on COOH-terminal half binding. Therefore, mild acidification probably promotes PIP₂ binding by directly altering the NH₂-terminal PIP₂ binding sites.

The significance of an increase in PIP₂ affinity described here depends on the PIP₂ concentration in the plasma membrane. This is difficult to estimate precisely because PIP₂ may be partitioned and sequestered. One estimate, based on PIP₂ accounting for 1% of plasma membrane lipid, suggests that the PIP₂ concentration in the plasma membrane of a spherical cell with a radius of 10 μm is 10 μM (44). In platelets, the PIP₂ concentration is estimated to be about 300 μM when averaged over the entire cell volume (internal and plasma membranes) (60), and PIP₂ concentration decreases by 30% following stimulation (16). Cytosolic [Ca²⁺] rises during agonist stimulation, and the 4–8-fold increase in CapG and gelsolin binding affinity described here is sufficiently large to promote their increased association with the plasma membrane despite a modest decrease in membrane PIP₂. The magnitude of the increase depends on the PIP₂ concentration before and after stimulation. Immunogold labeling studies show that 4 and 6.5% of gelsolin and CapG, respectively, are activated (31). Cytosolic [Ca²⁺] rises and PIP₂ level drops during agonist stimulation. This possibility is supported by experiments. Some actin-binding proteins are inhibited by PIP₂ (profilin, cofilin, capping protein), while others are activated by PIP₂ as well. It is significant that several pleckstrin homology (PH) domains, in vitro and in vivo experiments. In vitro, gelsolin stimulates and inhibits inositol-specific phospholipase C isoforms in a biphasic manner (29). Gelsolin stimulates phosphoinositide 3-OH-kinase (31), although we find that gelsolin and CapG also inhibit it. Gelsolin activates phospholipase D (30) in a PIP₂-dependent manner. Modest overexpression of CapG (28) or gelsolin has profound effects on phospholipase Cβ and phospholipase Cγ activated through two distinct receptor-mediated pathways.

In conclusion, these observations show that gelsolin and CapG binding to PIP₂ is selectively regulated by second messengers. This regulation provides an additional level of control above that of bulk change in PIP₂ content. Differential modulation and cross-talk between the PIP₂-binding proteins allow control to be exerted at multiple points in the signaling cascade.

Acknowledgments—We thank Drs. J. Albanesi, D. Hilgemann, and P. Thomas for helpful discussions and L. Segura for excellent technical assistance.

REFERENCES

1. De Camilli, P., Emr, S. D., McPherson, J. M., and Novick, P. (1996) Science 271, 1533–1539
2. Shaw, G. (1996) BioEssays 18, 55–46
3. Janmey, P. A. (1994) Annu. Rev. Physiol. 56, 169–191
4. Schafer, D. A., and Cooper, J. A. (1995) Annu. Rev. Cell Dev. Biol. 11, 497–518
5. Janmey, P. A., and Stossel, T. P. (1987) Nature 325, 362–364
6. Yu, F.-X., Johnston, P. A., Sudhof, T. C., and Yin, H. L. (1990) Science 250, 1413–1415
7. Schafer, D. A., Jennings, P. B., and Cooper, J. A. (1996) J. Cell Biol. 135, 169–179
8. Lassing, I., and Lindberg, U. (1985) Nature 314, 604–606
9. Yonezawa, N., Nishida, E., Iida, K., Yahara, I., and Sakai, H. (1990) J. Biol. Chem. 265, 8382–8386
10. Fukami, K., Furuhashi, K., Inagaki, M., Endo, T., Hatano, S., and Takenawa, T. (1992) Nature 359, 150–152
11. Gilmore, A. P., and Burridge, K. (1996) Nature 381, 521–535
12. Sun, H.-Q., Kwiatkowska, K., and Yin, H. L. (1995) Curr. Opin. Cell Biol. 7, 102–110
13. Hall, A. (1994) Annu. Rev. Cell Biol. 10, 31–54
14. Talas, K. F., Cantley, L. C., and Carpenter, C. L. (1995) J. Biol. Chem. 270, 17656–17659
15. Ren, X., Bokoch, G. M., Traynor-Kaplan, A., Jenkins, G. H., Anderson, R. A., and Schwartz, M. A. (1996) Mol. Biol. Cell 7, 435–442
16. Hartwig, J. H., Bokoch, G. M., Carpenter, C. L., Janmey, P. A., Taylor, L. A., Toker, A., and Stossel, T. P. (1995) Cell 82, 643–653
17. Chen, P., Murphy-Ullrich, J. E., and Wells, A. (1996) J. Cell. Biol. 134, 689–698
18. Appgar, J. R. (1995) Mol. Biol. Cell 6, 97–108
19. Dadabay, C. Y., Patton, E., Cooper, J. A., and Pike, L. J. (1991) J. Biol. Chem. 266, 1151–1156
20. Bengtsson, T., Rundquist, I., Stendahl, O., Wynn, M. P., and Andersson, T. (1988) J. Biol. Chem. 263, 17385–17389
21. Eberle, M., Traynor-Kaplan, A. E., Sklar, L. A., and Norgauer, J. (1990) J. Biol. Chem. 265, 16725–16728
22. Pike, L. J., and Casey, L. (1996) J. Biol. Chem. 271, 26453–26456
23. Hope, H. R., and Pike, L. J. (1996) Mol. Biol. Cell 7, 845–851
24. Glaser, M., Wanaski, S., Buser, C. A., Boguslavsky, V., Rashidzada, W., Morris, A., Rebecchi, M., Scarlata, S. F., Runnels, L. W., Prestwich, G. D., Chen, J., Aderem, A., Ahn, J., and McLaughlin, S. (1996) J. Biol. Chem. 271, 26187–26193
25. Yin, H. L., Zaner, K. S., and Stossel, T. P. (1994) J. Biol. Chem. 269, 9473–9479
26. Lamb, J. A., Allen, P. G., Tuan, B. Y., and Janmey, P. A. (1993) J. Biol. Chem. 268, 8899–9004
27. Young, C. L., Feierstein, A., and Southwick, F. S. (1994) J. Biol. Chem. 269, 13997–14002
28. Sun, H.-Q., Kwiatkowska, K., Wooten, D. C., and Yin, H. L. (1995) J. Cell Biol. 129, 147–156
29. Banno, Y., Nakashima, T., Kamada, T., Ebisawa, K., Nonomura, Y., and Nozawa, Y. (1992) J. Biol. Chem. 267, 6488–6494
30. Steed, P. M., Nagar, S., and Wennglock, L. F. (1996) Biochemistry 35, 5229–5237
31. Singh, S. S., Chauhan, A., Murakami, N., and Chauhan, V. P. (1996) Biochemistry 35, 16544–16549
32. Kwiatkowski, D. P., Stossel, T. P., Orkin, S. H., Mle, J. E., Colten, H. R., and Yin, H. L. (1996) Nature 323, 455–458
33. Yu, F.-X., Sun, H.-Q., Janmey, P. A., and Yin, H. L. (1992) J. Biol. Chem. 267, 14601–14605
34. Yu, F.-X., Zhou, D., and Yin, H. L. (1991) J. Biol. Chem. 266, 19269–19275
35. Sun, H.-Q., Wooten, D. C., Janmey, P. A., and Yin, H. L. (1994) J. Biol. Chem. 269, 9473–9479
36. Bradford, M. M. (1976) Annu. Rev. Biochem. 52, 248–254
37. Machesky, L. M., Goldschmidt-Clermont, P. J., and Pollard, T. D. (1990) Cell Regul. 1, 987–990
38. Goldschmidt-Clermont, P. J., Machesky, L. M., Baldassare, J. J., and Pollard, T. D. (1990) Science 247, 1575–1578

3 Sun, H.-Q., Lin, K.-M., and Yin, H. L. (1997) J. Cell Biol., in press.

4 P.-J. Lu, A.-L. Hsu, D.-S. Wang, H. Yan, H. L. Yin, and C.-S., Chen, manuscript in preparation.
39. Hummel, J. P., and Dreyer, W. J. (1962) Biochim. Biophys. Acta 63, 530–532
40. Ward, L. D. (1985) in Methods Enzymol. (Ward, L. D., ed) pp. 400–414, Academic Press, Inc., New York
41. Stinson, R. A., and Holbrook, J. J. (1997) Biochem. J. 311, 719–728
42. Hartwig, J. H., Chambers, K. A., and Stossel, T. P. (1989) J. Cell Biol. 108, 467–479
43. Lu, P., Shieh, W., Rhee, S. G., Yin, H. L., and Chen, C. (1996) Biochemistry 35, 14027–14034
44. Garcia, P., Gupta, R., Shah, S., Morris, A. J., Rudge, S. A., Scarlata, S., Petrova, V., McLaughlin, S., and Rebeiz, M. J. (1995) Biochemistry 34, 16228–16234
45. Zheng, J., Cahill, S. M., Lemmon, M. A., Fushman, D., Schlessinger, J., and Cowburn, D. (1996) J. Mol. Biol. 262, 14–21
46. Janmey, P. A., Iida, K., Yin, H. L., and Stossel, T. P. (1987) J. Biol. Chem. 262, 12228–12236
47. van Paridon, P. A., de Kruijff, B., Ouwerkerk, R., and Wirtz, K. W. (1986) Biochim. Biophys. Acta 877, 216–219
48. Chaponnier, C., Janmey, P. A., and Yin, H. L. (1986) J. Cell Biol. 103, 1473–1481
49. Yin, H. L., Iida, K., and Janmey, P. A. (1988) J. Cell Biol. 106, 805–812
50. Janmey, P. A., Lamb, J., Allen, P. G., and Matsudaira, P. T. (1992) J. Biol. Chem. 267, 11818–11823
51. Kwiatkowski, D., Janmey, P. A., Mole, J. E., and Yin, H. L. (1985) J. Biol. Chem. 260, 15222–15238
52. Cunningham, C. C., Stossel, T. P., and Kwiatkowski, D. J. (1991) Science 251, 1233–1236
53. Witke, W., Sharpe, A. H., Hartwig, J. H., Azuma, T., Stossel, T. P., and Kwiatkowski, D. J. (1995) Cell 81, 41–51
54. Arora, P. D., and McCallioch, C. A. G. (1996) J. Biol. Chem. 271, 20516–20523
55. Barkalow, K., Witke, W., Kwiatkowski, D. J., and Hartwig, J. H. (1996) J. Cell Biol. 96, 389–399
56. Kwiatkowski, D., Janmey, P. A., and Yin, H. L. (1989) J. Cell Biol. 108, 1717–1726
57. McLaughlin, P. J., Gooch, J. T., Mannherz, H.-G., and Weeds, A. G. A. (1993) Nature 364, 685–692
58. Van Troys, M., Dewitte, D., Goethals, M., Vandekerckhove, J., and Ampe, C. (1996) FEBS Lett. 396, 191–193
59. Selve, N., and Wegner, A. (1987) Eur. J. Biochem. 168, 111–115
60. Rittenhouse, S. E., and Sasson, J. P. (1985) J. Biol. Chem. 260, 8657–8660
Gelsolin Binding to Phosphatidylinositol 4,5-Bisphosphate Is Modulated by Calcium and pH

Keng-Mean Lin, Elizabeth Wenegieme, Pei-Jung Lu, Ching-Shih Chen and Helen L. Yin

J. Biol. Chem. 1997, 272:20443-20450.
doi: 10.1074/jbc.272.33.20443

Access the most updated version of this article at http://www.jbc.org/content/272/33/20443

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 58 references, 33 of which can be accessed free at http://www.jbc.org/content/272/33/20443.full.html#ref-list-1