Syndecan-4 Proteoglycan Cytoplasmic Domain and Phosphatidylinositol 4,5-Bisphosphate Coordinately Regulate Protein Kinase C Activity*

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Eok-Soo Oh, Anne Woods, Ssang-Taek Lim, Anne W. Theibert‡, and John R. Couchman§

From the Department of Cell Biology, Cell Adhesion and Matrix Research Center and ‡Department of Neurobiology, University of Alabama, Birmingham, Alabama 35294

Phosphatidylinositol 4,5-bisphosphate (PIP₂) is involved in the organization of the actin cytoskeleton by regulating actin-associated proteins. The transmembrane heparan sulfate proteoglycan syndecan-4 also plays a critical role in protein kinase C (PKC) signaling in the formation of focal adhesions and actin stress fibers. The cytoplasmic domain of syndecan-4 core protein directly interacts with and potentiates PKC activity, and it can directly interact with the phosphoinositide PIP₂. We, therefore, investigated whether the interaction of inositol phosphates and inositol phospholipids with syndecan-4 could regulate PKC activity. Data from in vitro kinase assays using purified PKCαβγ show that in the absence of phosphatidylserine and diolein, PIP₂ increased the extent of autophosphorylation of PKCαβγ and partially activated it to phosphorylate both histone III-S and an epidermal growth factor receptor peptide. This activity was dose-dependent, and its calcium dependence varied with PKC isotype/source. Addition of the cytoplasmic syndecan-4 peptide, but not equivalent syndecan-1 or syndecan-2 peptides, potentiated the partial activation of PKCαβγ by PIP₂, resulting in activity greater than that observed with phosphatidylserine, diolein, and calcium. This study indicates that syndecan-4 cytoplasmic domain may bind both PIP₂ and PKCα, localize them to forming focal adhesions, and potentiate PKCα activity there.

The control of cellular adhesion status is complex, involving several signaling mechanisms (1–4). Phosphatidylinositol 4,5-bisphosphate (PIP₂) plays important roles in the organization of the actin cytoskeleton. PIP₂ may control actin polymerization by regulating the binding of actin-binding proteins such as profilin and gelsolin to actin (5, 6). PIP₂ may also interact with α-actinin and vinculin (7) and regulate their association with the cytoskeleton (8). The level of PIP₂ decreases upon detachment of cells from the substratum and increases upon reattachment to fibronectin (1). The difference in the levels of PIP₂ is probably due to different rates of phosphorylation of phosphatidyl 4-phosphate to PIP₂ by phosphatidylinositol 4-phosphate 5-kinase. Phosphatidylinositol 4-phosphate 5-kinase is stimulated 3–4-fold by adhesion of cells to fibronectin (1), probably through interactions with the small GTP-binding proteins Rac and Rho, the latter of which has also been implicated in the regulation of assembly of actin stress fibers and focal adhesions (9–13).

PIP₂ may enter different pathways in signal transduction. It can be hydrolyzed by phospholipase Cγ to generate two intracellular messengers: inositol 1,4,5-triphosphate, which mobilizes Ca²⁺, and diacylglycerol, which is a physiological activator of protein kinase C (PKC). It can be further phosphorylated by phosphatidylinositol 3-kinase to generate phosphatidylinositol 3,4,5-triphosphate (PIP₃), which has been proposed to regulate numerous activities including cytoskeletal organization (14) and vesicle trafficking (15). PIP₂ can also be dephosphorylated via the 5-phosphatase to phosphatidylinositol 4-phosphate (16). PKCα may also directly activate several proteins including PKC. PKCα is a potent activator of conventional PKC isoforms (α, β, βII, and γ) in the presence of phosphatidylserine (PS) and calcium (17–19). Indeed, PKCα is more potent than diacylglycerol in stimulating PKC in vitro (20), and it stimulates the translocation of conventional PKC from the soluble to the particulate fraction (18). Thus, PIP₂ may itself be a primary activator of PKC in vivo, both activating it and inducing its association with the plasma membrane (19, 21).

PKC activity is needed for matrix-induced cell spreading (22) and for the later stage of focal adhesion assembly (23). Cell surface heparan sulfate proteoglycans have critical role(s) in PKC signaling in focal adhesion and actin stress fiber formation (23–26). Cell attachment and spreading can be promoted through integrin interactions with the cell binding domain of fibronectin (23). However, normal anchorage-dependent fibroblasts require an additional signal(s) to form focal adhesions, which occur after binding of a heparin binding domain of fibronectin (23). Normal anchorage-dependent fibroblasts require an additional signal(s) to form focal adhesions, which occur after binding of a heparin binding domain of fibronectin or a peptide from this domain to a cell surface heparan sulfate proteoglycan (23–26). These interactions may stimulate PKC activity, since PKC inhibitors prevent focal adhesion formation, and pharmacological activation of PKC can substitute for stimulation through heparin binding moieties (23). Syndecan-4 is one of four mammalian transmembrane heparan sulfate proteoglycans that share a high degree of similarity, and it is selectively concentrated in focal adhesions in numerous cell types (27). It may transduce the signal(s) generated on binding of heparin binding moieties to cells. A unique region of its cytoplasmic domain (LGKKPIYKK) can potentiate PKCα activity in vitro, and PKC interacts with its core protein in vivo, and with synthetic peptides of the LGKKPIYKK sequence (28). The interactions between PIP₂ and several PIP₂-
binding proteins may be through their pleckstrin homology domains (20, 29–32), where two lysine residues, which end a β1 strand at the turn, interact with the 4- and 5-phosphates of the inositol head group of PIP₂ (31). The cytoplasmic sequence of syndecan-4 bears some similarity to pleckstrin homology domains, and the LGKKPIYKK peptide from the cytoplasmic domain of syndecan-4 can interact with the phosphoinositides PIP₂ and inositol hexaphosphate (IP₆). Since syndecan-4 can bind PIP₂ and activate PKC, we investigated whether PIP₂ and syndecan-4 act synergistically to activate PKC, representing an alternative pathway to those previously described.

EXPERIMENTAL PROCEDURES

Materials—Synthetic peptides corresponding to the whole cytoplasmic domain of syndecan-4 (4L) and to the central, unique region of syndecan-4 (4V), -2 (2V), or -1 (1V), a peptide having the scrambled sequence of 4V (Scr), and one where the proline was substituted with alanine (4VPA) were synthesized and sequenced by the University of Alabama at Birmingham Comprehensive Cancer Center Peptide Synthesis and Analysis Shared Facility (Table I). PKCaβγ purified from rabbit brain and recombinant PKCa were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). An alternate source of recombinant PKCa was Life Technologies, Inc., and similar results were obtained for both. [γ-32P]ATP was obtained from NEN Life Science Products. The peptide representing the phosphorylation site in the epidermal growth factor (EGF) receptor and P81 phosphocellulose paper were obtained from Biomol Research (Plymouth Meeting, PA) and Whatman (Fairfield, NJ), respectively. Phosphoinositides PIP₂, IP₃, and inositol tetraphosphate (IP₆), histone III-S, myelin basic protein, and other chemicals were purchased from Sigma. PIP₂ was synthesized by Dr. Roy Gigg (National Institute of Medical Research, London, UK).

In Vitro PKC Assay—The standard reaction mixture (total 20 μl) contained 50 mM HEPES (pH 7.3), 3 mM magnesium acetate, PKCaβγ (3 ng) or PKCa (1 ng), and 4 μg of histone III-S or myelin basic protein as a substrate. 0.2 mg/ml PS and 0.02 mg/ml diolein (DL) were added as required, and different amounts of phosphoinositides were added as detailed in the text. CaCl₂ was added as indicated in the figure legends and text, and 0.25 mg/ml each of synthetic peptides were present. Reactions were started by the addition of 200 μM ATP (0.5 mCi of [γ-32P]ATP). After 10 min at room temperature, the reaction was stopped by adding SDS-polyacrylamide gel electrophoresis sample buffer and separated by 20% SDS-polyacrylamide gel electrophoresis, and phosphorylated histone III-S or myelin basic protein was detected by autoradiography and quantified by Bio Rad Model GS-670 imaging densitometer. In assays using 0.1 mg/ml EGF receptor peptide of the sequence RKRLRTLRL as an alternate substrate (33), the reaction was stopped by spotting the whole reaction mixture onto phosphocellulose filters (Whatman, p81, 2.1 cm) and then washing these into 75 mM phosphoric acid. Filters were washed 3 × 10 min, immersed in 95% ethanol for 5 min, dried, and counted with 4 ml of scintillation mixture in a scintillation counter (Wallac Model 1419).

Autophosphorylation of PKC αβγ—Reaction mixtures prepared as described above with 50 μM PIP₂ or IP₆ in the absence of any activators (PS/DL, calcium) or substrate were incubated at 30 °C for 5 min and stopped by the addition of SDS sample buffer and heating to 95 °C for 5 min. Proteins were separated by 7.5% SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

RESULTS

PIP₂ and IP₆ Can Partially Activate PKCaβγ—We first investigated whether phosphoinositides could elevate the activity of a mixture of PKCaβγ in vitro. In the absence of PS and DL, phosphoinositides increased the activity of PKCaβγ to phospholipase histone III-S (Fig. 1A) or myelin basic protein (data not shown). PIP₂ addition resulted in the highest level of PKCaβγ activity (approximately 4-fold over control levels; compare lanes 1 and 2). The same concentrations of PIP₃ and IP₆ (lanes 3 and 5, respectively) also increased activity (approximately 3-fold), whereas the effect of inositol tetraphosphate (lane 4) was not significant. The activation of PKCaβγ by PIP₂ was approximately 60% of the maximal activity by conventional stimulation (refer to Fig. 6A) by PS/DL (0.2 mg/ml PS and 0.02 mg/ml DL) and calcium. When PS/DL was present, PIP₃, IP₃, IP₆, and IP₇ had no significant effect on the ability of PKCaβγ to phosphorylate histone III-S (data not shown). The effect of IP₆ on the phosphorylation of histone III-S by PKCaβγ was dose-dependent and maximal at 50 μM IP₆ (Fig. 1B). Stimulation of PKCaβγ by PIP₂ was also dose-dependent, with half maximal stimulation at 30 μM and a maximum at 50 μM PIP₂ (Fig. 1C).

Calcium Dependence of Activation—Since PKCaβγ are known as calcium-dependent enzymes and PIP₂ interacts with PKC through its regulatory domain (18, 34), we investigated whether calcium affected the increased activity of PKCaβγ in the presence of PIP₂ and IP₆ (Fig. 2). In contrast to that observed with PS/DL, no effect was seen at physiological intracellular calcium levels (50–100 nM; Refs. 35–37) on the activation of PKCaβγ by either PIP₂ or IP₆, indicating calcium independence. Minor increases in phosphorylation were seen with PIP₂ and IP₆ at 1–30 μM calcium, but at concentrations above 50 μM, calcium significantly inhibited the activity. This is consistent with previous reports demonstrating the inhibition by calcium of PIP₂-induced potentiation of the activity of PKCβ₁, -α, and -γ in mixed micelles (38). In contrast, Phosphor Imager analysis of autoradiographs with recombinant PKCa indicated calcium dependence, with 25 μM causing a 2.7- and 8.8-fold increase in activity in the absence of PS/DL (Fig. 3A) relative to the untreated control (Fig. 3B).

TABLE I

| Peptide | Sequence |
|---------|----------|
| 4L      | RMKKKDEGYSQGKPYVKAPTNEFYA |
| 4V      | LGIKFQYYK |
| 4PA     | LGKAIYKK |
| Scr     | KRYRKIPLK |
| 2V      | CGERKRESSAAAYQK |
| 1V      | CANGGAYQ |

**FIG. 1. Phosphoinositide activation of PKCaβγ to phosphorylate histone III-S in the absence of phospholipid. A, phosphoinositides (50 μM) were added to assays in the absence of both calcium and PL, and autoradiographs of phosphorylated histone III-S (HIS, inset) were quantified by densitometer. Values shown are mean ± S.E. (n = 3). Activation was dose-dependent with both IP₃ (B) and PIP₂ (C).**
3.5-fold increase in phosphorylation of histone III-S in the presence of PIP₂ and IP₆, respectively (not shown, but see Fig. 6).


PIP₂ but Not IP₆, Directly Activates PKCαβγ—Phosphoinositides such as PIP₂ and IP₆ are highly negatively charged, whereas histone III-S and myelin basic protein are positively charged. It was possible, therefore, that increased phosphorylation of substrate by PKCαβγ was due to either increased PKCαβγ activity or increased accessibility of the substrate to PKCαβγ. We therefore investigated whether PIP₂ or IP₆ could increase autophosphorylation of PKCαβγ in the absence of PS/DL (Fig. 3). PIP₂ increased autophosphorylation of PKCαβγ over that seen in the absence of PIP₂ (compare lanes 1 and 2). However, autophosphorylation of PKCαβγ in the presence of IP₆ was not increased (compare lanes 3 and 4). Thus, IP₆ may increase PKCαβγ phosphorylation of basic substrates by charge interactions that increase substrate accessibility. In contrast, PIP₂ may directly affect PKCαβγ. To substantiate this hypothesis, PKCαβγ assays were performed in the presence of PIP₂ or IP₆ using a peptide substrate from the EGF receptor (Fig. 4). PIP₂ increased PKCαβγ phosphorylation of this substrate approximately 3-fold (compare lanes 1 and 3), whereas no increase was seen with IP₆ (compare lanes 1 and 9). Although this activation was less than that seen using histone III-S as substrate, it was statistically significant (p < 0.001). As seen with histone III-S phosphorylation, PIP₂, but not IP₆, also increased the phosphorylation of the EGF receptor peptide approximately 2.5-fold (compare lanes 1 and 5).

Syndecan-4 Further Potentiates PKCαβγ Activity Induced by PIP₂ but Not by Other Phosphoinositides—Our previous studies showed that syndecan-4 could directly activate recombinant PKCα and potentiate its activation by phospholipid through a defined region of the syndecan-4 cytoplasmic domain (28). Further experiments determined whether syndecan-4 could also affect the PIP₂-induced activation of PKCαβγ using EGF receptor peptide (Fig. 4) or histone III-S (Fig. 5) as substrates. The results for both were similar. Peptide 4V from the cytoplasmic domain of syndecan-4 potentiated the activity of PKCαβγ to phosphorylate the EGF receptor peptide in the presence of PIP₂ from approximately 3-fold to 7-fold (Fig. 4, compare lanes 3 and 4 with 1). It had no effect, however, on activity in the presence of PIP₆ (compare lanes 5 and 6), IP₄ (compare lanes 7 and 8), or IP₆ (compare lanes 9 and 10). Similar results were obtained monitoring histone III-S phosphorylation (Fig. 5A). PIP₂ alone increased the activity of PKCαβγ to phosphorylate histone III-S approximately 5-fold (Fig. 5A, compare lanes 1 and 3). Peptide 4V in the absence of inositol lipid or phospholipid showed a direct activation, as seen previously (28), but to a smaller (approximately 1.5-fold) extent (lane 2). The presence of both PIP₂ and 4V potentiated the activation of PKCαβγ to approximately 11 times that of control levels (Fig. 5A, compare lanes 3 and 4 with 1). However, 4V did not further increase phosphorylation of histone III-S by PKCαβγ in the presence of IP₆ (Fig. 5B, compare lanes 3 and 4), again suggesting that IP₆ and PIP₂ act through different mechanisms.

To investigate whether the potentiation of PIP₂-induced PKC activity by syndecan-4 could be significant in vivo, we compared the maximal activity of PKCαβγ or PKCα in the presence of both PIP₂ and syndecan-4 peptide with that of PKC induced by other physiological PKC phospholipid activators (Fig. 6). As seen previously (28), basal levels of phosphorylation were detected in the absence of phospholipid and calcium (Fig. 6A, lane 1). PS/DL in the presence of 750 μM calcium normally induced maximal phosphorylation (lane 2), as seen in our assays (28) and by others (39). In the presence of 50 μM PIP₂ and the syndecan-4 peptide 4L or 4V, there was even greater activity of PKCαβγ, even in the absence of PL and calcium (Fig. 6A,
We have previously shown that a peptide sequence from the cytoplasmic domain of syndecan-4 (4V), the unique regions of the cytoplasmic domain of syndecan-4, but not those of syndecan-1 or syndecan-2, can potentiate PKCαβ activity by PKCβ2. 

**DISCUSSION**

A variety of evidence implicates PKC activity in cell-cell and cell-matrix interactions. In most cases, the isoform of PKC is unknown, though a role for PKCα emerges from its presence in focal adhesions of normal, but not transformed, cells (45, 46). PKCαβ have been characterized as calcium and phospholipid-dependent isozymes, requiring both cofactors for activity. We have previously shown that a peptide sequence from the cytoplasmic domain of syndecan-4 can directly activate PKCα. In the absence of PS/DL and calcium, a modest increase is observed (1.5-fold), whereas addition of syndecan-4 peptide in the presence of PS/DL/Ca2+ produces a large en-

**FIG. 5.** The effect of syndecan-4 peptides on the increased phosphorylation of histone III-S (HIS) by PKCαβ in the presence of 50 μM PIP2 (A) and IP6 (B). In vitro PKC assays were performed in the absence of PS/DL and calcium. Results are the mean ± S.E. (n = 3) of densitometric analysis of autoradiograms, a representative one of which is inset.

Compare lanes 3 and 4 with 2). Again PIP2 alone induced some activation of PKCαβ in the absence of PS/DL, peptide, or calcium (lane 5). With recombinant PKCα (Fig. 6B), similar results were seen, although low levels of calcium were required. Calcium alone did not activate PKCα (lane 1) but peptide 4L (lane 2) or PIP2 (lane 3) did, and a further increase was seen in the presence of both 4L and PIP2 (lane 4). An additional control was that the altered 4V peptide (proline substituted with alanine), which had no effect in potentiating PS/DL-mediated PKCα activity (28), also had no effect on PIP2-mediated activation (Fig. 6C). Neither 25 μM calcium (lane 1) nor the 4PA peptide ± calcium (lanes 2 and 3) activated PKCα. PIP2 activation of PKCα was dependent on the presence of 25 μM calcium (compare lanes 4 and 5). Again, 4PA peptide did not increase the activity seen with PIP2 alone ± calcium (compare lanes 6 and 7 with lanes 4 and 5). Lane 8 shows the maximal activity of PKCα in the presence of PS/DL and 750 μM calcium. Activation of recombinant PKCα by PIP2 appears to be dependent on at least 25 μM calcium (Fig. 6C, lanes 4 and 5), whereas that of purified PKCαβ is not (Fig. 6A, lane 5). This was confirmed (Fig. 6D) by the fact that potentiation of PIP2-induced PKCαβ phosphorylation of histone III-S (lane 1) by the syndecan 4L (lanes 2–4) and 4V (lanes 5–7) peptides was virtually unaffected by the presence of 10 μM (lanes 3 and 6) or 100 μM (lanes 4 and 7) calcium or even 1 mM EGTA (lanes 2 and 5).

**The Effect on PKC Activity Is Unique to a Syndecan-4 Cytoplasmic Sequence—**Since all syndecans have high homology in 2 regions of the cytoplasmic domain with intervening variable sequences (28), we determined whether the potentiation of PIP2-induced PKC activity was unique to syndecan-4 (Fig. 7). We used synthetic peptides corresponding to the whole cytoplasmic domain of syndecan-4 (4L), the unique regions of the cytoplasmic domain of syndecans-4 (4V), -2 (2V) or -1 (1V), and a peptide where the normal sequence of 4V was scrambled (Scr) in assays monitoring phosphorylation of histone by PKCαβ in the presence of PIP2. Synthetic peptides 4L (lane 1) and 4V (lane 2) potentiated PIP2-induced activity of PKCαβ, but Scr (lane 4) and 2V (lane 5) or 1V (lane 6) had no effect. Thus, the cytoplasmic domain of syndecan-4, but not those of syndecan-1 or syndecan-2, can potentiate PKCαβ activity by PKCβ2.

**FIG. 6.** Effect of syndecan-4 peptides and calcium on PIP2-induced activation of PKCαβ and recombinant PKCα. A, autoradiographs show the basal level of phosphorylation by PKCαβ of histone III-S in the absence of PS/DL (PL) and 750 μM calcium (lane 1) and normally maximal phosphorylation in their presence (lane 2). Phosphorylation by PKCαβ is even higher in the presence of PIP2 and peptide 4L (lane 3) or 4V (lane 4), with lower levels in the presence of PIP2 alone (lane 5). B, recombinant PKCα is not activated in the presence of 25 μM calcium (lane 1), but this is sufficient to allow activation by peptide 4L (lane 2) or PIP2 (lane 3) and potentiation of activity with a combination of PIP2 and 4L peptide (lane 4). C, peptide 4PA (proline substituted by alanine) does not activate recombinant PKCα ± 25 μM calcium (compare lane 1 with 2 and 3). PIP2 activation of PKCα requires the presence of 25 μM calcium (compare lanes 4 and 5) and is not increased in the presence of peptide 4PA (lanes 6 and 7). Maximal phosphorylation is seen in the presence of PS/DL (PL) and 750 μM calcium (lane 8). D, the activation of PKCαβ by PIP2 and 4L or 4V is calcium-independent, since 1 mM EGTA has little effect (compare lanes 3 and 6 with 2 and 5), and high calcium (100 μM) does not increase activation (lanes 4 and 7).
hancement of the PS/DL/Ca²⁺-stimulated activities, leading to an 11-fold stimulation over basal activity (28). Similar to published reports, the present studies show that a phosphoinositide previously implicated in transmembrane signaling (16, 47), PIP₂, partially activates PKC in the absence of PS/DL, and this is increased by the syndecan-4 peptide.

Previous studies by Toker et al. (48) have investigated the activation of PKC isotypes by phosphoinositides. In the presence of 10 μM phosphatidylycerine and 40 μM phosphatidylethanolamine, most phosphoinositides, including PIP₂, did not significantly activate PKCα. They also failed to detect any significant activation of PKCα by 10 μM PIP₂ in the absence of phospholipid (48). Our experiments show that PKCαβγ requires 50 μM PIP₂ for maximum activation in the absence of PS/DL to phosphorylate three different substrates: histone III-S, myelin basic protein, and the EGF receptor peptide. In platelets, the concentration of PIP₂ may be as high as 140–240 μM (49), supporting physiological activation of PKC by PIP₂.

In contrast to published reports, we report here that there is little or no calcium dependence for PIP₂ stimulation of PKCαβγ in the presence of 50 μM PIP₂ and absence of PS/DL, although activation of recombinant PKCα is dependent on low levels of calcium (25 μM). This may be due to differences in preparation of PKCαβγ and recombinant PKCα, leading to varying degrees of phosphorylation (50). The phosphorylation status of intracellular PKC isoforms is not clear. IP₃, which activates intracellular calcium channels, is known to be produced by the hydrolysis of PIP₂ by phospholipase Cγ after ligand binding to receptors (8). It is, however, not entirely resolved whether or not calcium transients accompany integrin-mediated activities, leading to an extracellular matrix molecule such as fibronectin, PIP₂ levels increase, and this may be an important regulatory factor for actin polymerization and stress fiber and focal adhesion formation (11, 12). In addition, PIP₂ and PKC activation are both required for focal adhesion and stress fiber formation (24, 52).

We have previously shown (28) that PKCα copatches when syndecan-4 is patched by the addition of ectodomain antibodies to spreading fibroblasts, and they can be coimmunoprecipitated. Moreover, PKCα, once activated by phospholipid or phospholipid ceramide, can interact in vitro with the cytoplasmic domain of syndecan-4 through the sequence LGKPIYKK, and this potentiates PKCα activity (28). A synthetic peptide of the same sequence also interacts with PIP₂, and this promotes oligomerization of the syndecan-4 cytoplasmic domain (53). The fact that PIP₂ in the presence of syndecan-4 can together give rise to high PKC activity suggests that ternary interactions between PIP₂, syndecan-4 cytoplasmic domain, and PKCα may be the most relevant activation of PKCα in the regulation of focal adhesion and stress fiber formation. This would not require an involvement of any other second messenger signaling mechanism such as phospholipase Cγ-dependent calcium fluxes or diacylglycerol production. However, it is not yet known whether interactions of two of the three components, syndecan-4, PIP₂, and PKCα, influences further binding of the third to form a ternary complex. Our previous data suggest that syndecan-4 core protein interacts with the catalytic domain of PKCα (28), whereas PIP₂ probably binds the regulatory domain of PKCα (19, 24) even more strongly than diacylglycerol (20).

Both PKCα and PIP₂ appear to interact with the same region of syndecan-4, namely the central V region (LGKPIYKK). The binding of PIP₂ and PKC to this region is not mutually exclusive. Although PIP₂ or 4V alone modestly up-regulate PKC-mediated phosphorylation of substrates, the addition of both agents leads to a synergistic stimulation of kinase activity. In addition, only oligomeric forms of syndecan-4 stimulate PKC activity (53).

Our binding data indicates that IP₆ can also interact with syndecan-4. However, in contrast to PIP₂, IP₆ could activate PKCαβγ only when phosphorylating histone III-S as a substrate, not when using the EGF receptor peptide as a substrate. Experiments examining the autophosphorylation of PKCαβγ indicate that IP₆ may not directly activate the enzymes but rather increase the apparent activity by changing substrate accessibility. Since most experiments investigating PKC activation by phosphoinositides have used highly basic substrates including myelin basic protein, any increased phosphorylation seen may be due to either or both increased activity or substrate accessibility. One further experiment also supports the hypothesis that PIP₂ rather than IP₆ is the active participant in a signaling complex. Although IP₆ can also bind the syndecan-4 peptide, PIP₂, but not IP₆, will promote the oligomerization of full-length syndecan-4 cytoplasmic domain (4L), with a concomitant stimulation of kinase activity of PKCαβγ by the oligomeric peptide (53).
REFERENCES

1. Schwartz, M. A. (1992) Trends Cell Biol. 2, 304–308
2. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Dev. Biol. 11, 549–599
3. Parsons, J. T. (1996) Curr. Opin. Cell Biol. 8, 146–152
4. Yamada K. M., and Miyamoto, S. (1995) Curr. Opin. Cell Biol. 7, 681–688
5. Machelsky L. M., and Pollard, T. D. (1995) Trends Cell Biol. 3, 381–385
6. Schafer, D. A., and Cooper, J. A. (1995) Annu. Rev. Cell Dev. Biol. 11, 497–518
7. Fukami, K., Endo, T., Imamura, M., and Takenawa, T. (1994) J. Biol. Chem. 269, 1518–1522
8. Gilmore, A. P., and Burridge, K. (1996) Nature 381, 531–535
9. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
10. Zhang, J., King, W. G., Dillon, S., Hall, A., Feig, L., and Rittenhouse, S. E. (2016) Cell 167, 139–149
11. Chong, L. D., Kaplan, A. T., Bokoch, G. M., and Schwartz, M. A. (1994) Cell 76, 765–775
12. 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
13. Tolias, K. F., Cantley, L. C., and Carpenter, C. L. (1995) J. Biol. Chem. 270, 17656–17659
14. Wennstrom, S., Hawkins, P. T., Cooke, F., Hara, K., Yonezawa, K., Kasuga, M., Jackson, T., Claesson-Welsh, L., and Stephens, L. R. (1994) Curr. Biol. 4, 385–393
15. Shepherd, P. S., Reaves, B. J., and Davidson, H. W. (1996) Trends Cell Biol. 6, 92–97
16. Directa, N., and Irvine, R. F. (1995) Cell 80, 269–278
17. Chauhan, V. S. P. (1990) FEBS Lett. 272, 99–102
18. Chauhan, A., Brockerhoff, H., Winiarski, H. M., and Chauhan, V. S. P. (1991) Arch. Biochem. Biophys. 287, 283–299
19. Lee, M., and Bell, R. M. (1991) Biochemistry 30, 1041–1049
20. Pap, E. H. W., Bastiaens, P. I. H., Vorster, J. W., van den Berg, P. A. W., van Hooft, J., Snoek, O. T., Wirtz, K. W. A., and Visser, A. J. W. G. (1993) Biochemistry 32, 13310–13317
21. Bell, R. M., and Burns, D. J. (1993) J. Biol. Chem. 268, 4661–4664
22. Vuori, K., and Russel, E. (1993) J. Biol. Chem. 268, 21459–21462
23. Woods, A., and Couchman J. R. (1992) J. Cell Sci. 101, 277–290
24. Woods, A., Couchman, J. R., Johansson, S., and Hook, M. (1986) EMBO J. 5, 665–670
25. Woods, A., and Couchman J. R. (1992) Adv. Exp. Med. Biol. 313, 87–96
26. Woods, A., McCarthy, J. B., Puchert, L. T., and Couchman, J. R. (1993) Mol. Biol. Cell 4, 605–613
27. Woods, A., and Couchman J. R. (1994) Mol. Biol. Cell 5, 183–192
28. Oh, E. S., Woods, A., and Couchman J. R. (1997) J. Biol. Chem. 272, 8133–8136
29. Janney, P. A. (1995) Chem. Biol. 2, 61–65
30. Harlan, J. E., Hajduk, P. J., Yoon, H. S., and Fesik, S. W. (1994) Nature 371, 168–170
31. Harlan, J. E., Yoon, H. S., Hajduk, P. J., and Fesik, S. W. (1995) Biochemistry 34, 8639–8644
32. Sohn, K. H., Chen, J., Koblin, K. S., Bray, P. F., and Goldschmidt-Clermont, P. J. (1995) J. Biol. Chem. 270, 21114–21120
33. Hunter, T., Ling, N., and Cooper, J. A. (1994) Nature 311, 480–483
34. Chauhan, A., Chauhan, V. S. P., Deshmukh, D. S., and Brockerhoff, H. (1999) Biochemistry 28, 4952–4959
35. Schwartz, M. A. (1993) J. Cell Biol. 120, 1003–1019
36. Takahashi, Y., Yoshida, T., and Takashima, S. (1992) J. Gerontol. 47, 65–70
37. Banyard, M. R., and Tellam, R. M. (1985) Br. J. Cancer 51, 761–766
38. Palmer, R. H., Dekker, L. V., Wocjolski, R., Le Good, J. A., Gigg, R., Parker, P. J. (1995) J. Biol. Chem. 270, 22412–22416
39. Kikkawa, U., Takai, Y., Minakuchi, S., Inohara, S., and Nishizuka, Y. (1982) J. Biol. Chem. 257, 13341–13348
40. Lewis, J. M., Cherezov, D. A., and Schwartz, M. A. (1996) J. Cell Biol. 134, 1323–1332
41. Chun, J. S., and Jacobson, B. S. (1993) Mol. Biol. Cell 4, 271–281
42. Somers, C. E., and Mosher, D. F. (1993) J. Biol. Chem. 268, 22277–22280
43. Ginsberg, M. H., Du, X., and Flor, E. F. (1992) Curr. Opin. Cell Biol. 4, 766–771
44. Herbert, J. M. (1993) Biochem. Pharmacol. 45, 527–537
45. Hyatt, S. L., Klauck, T., and Jaken, S. (1990) Mol. Carcinog. 4, 45–53
46. Jaken, S., Leach, K., and Klauck, T. (1989) J. Cell Biol. 109, 697–704
47. Majerus, P. W. (1992) Annu. Rev. Biochem. 61, 225–250
48. Toker, A., Meyer, M., Reddy, K. K., Fals, J. R., Aneja, R., Aneja, S., Parra, A., Burns, D. J., Ballas, L. M., and Cantley, L. C. (1994) J. Biol. Chem. 269, 32358–32367
49. Machelsky, L., Goldschmidt-Clermont, P. J., and Pollard, T. (1990) Cell Regul. 1, 957–960
50. Bernincin, P., and Parker, P. J. (1997) J. Biol. Chem. 272, 3544–3549
51. Leavesley, D. I., Schwartz, M. A., Rosenfeld, M., and Cheresh, D. A. (1993) J. Cell Biol. 121, 163–170
52. Bacic, P. C., and Gostinck, P. F. (1995) Mol. Biol. Cell 6, 1503–1513
53. Oh, E. S., Woods, A., and Couchman, J. R. (1997) J. Biol. Chem. 272, 11805–11811
54. Bernfield, M., Kokenyesi, R., Kato, M., Hinkes, M. T., Spring, J., Gallo, R. L., and Lose, E. J. (1992) Annu. Rev. Cell Biol. 8, 365–393