Regulation of G Protein-coupled Receptor Kinases by Caveolin*

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G protein-coupled receptor kinases (GRKs) have been principally characterized by their ability to phosphorylate and desensitize G protein-coupled receptors. However, recent studies suggest that GRKs may have more diverse protein/protein interactions in cells. Based on the identification of a consensus caveolin binding motif within the pleckstrin homology domain of GRK2, we tested the direct binding of purified full-length GRK2 to various glutathione S-transferase-caveolin-1 fusion proteins, and we discovered a specific interaction of GRK2 with the caveolin scaffolding domain. Interestingly, analysis of GRK1 and GRK5, which lack a pleckstrin homology domain, revealed in vitro binding properties similar to those of GRK2. Maltose-binding protein caveolin and glutathione S-transferase-GRK fusion proteins were used to map overlapping regions in the N termini of both GRK2 and GRK5 that appear to mediate conserved GRK/caveolin interactions. In vivo association of GRK2 and caveolin was suggested by co-fractionation of GRK2 with caveolin in A431 and NIH-3T3 cells and was further supported by co-immunoprecipitation of GRK2 and caveolin in COS-1 cells. Functional significance for the GRK/caveolin interaction was demonstrated by the potent inhibition of GRK-mediated phosphorylation of both receptor and peptide substrates by caveolin-1 and -3 scaffolding domain peptides. These data reveal a novel mode for the regulation of GRKs that is likely to play an important role in their cellular function.

G protein-coupled receptor kinases (GRKs) phosphorylate the agonist-activated form of G protein-coupled receptors that in turn promotes the high affinity binding of arrestins (1). This process functions to both uncouple the receptor from the G protein and to promote receptor internalization via clathrin-coated pits. The ability to regulate interaction of GRKs with the plasma membrane where receptor substrates reside. Recent studies have provided novel information regarding the function and cellular localization of GRKs. For example, it was shown that GRK2 can traffic along with β2-adrenergic receptors to the endosome following receptor activation (2). Mayor and co-workers (3) have also demonstrated the association of GRK2 with microsomes that appears to be mediated via an unidentified GRK2-binding protein. In addition, we and others (4–6) have recently demonstrated novel interactions between GRKs and the cytoskeleton. Collectively, these studies suggest that the function and regulation of GRKs may involve diverse protein/protein interactions.

Caveolae represent distinct cholesterol- and glycosphingolipid-enriched plasma membrane and vesicular structures in cells that function in a variety of cellular processes including endothelial transcytosis and potocytosis (7). Caveolin, a 22–24 kDa integral membrane protein composed of cytoplasmic N and C termini and a central intramembrane domain, is thought to be a major structural component of caveolae (7). A 20-amino acid juxtamembrane region (the scaffolding domain) within the N-terminal domain has been shown to mediate the association of caveolin with other proteins (8). Recently, a wide variety of signaling molecules have been shown to associate with caveolin leading to the hypothesis that caveolae may serve as cell-surface microdomains that concentrate and organize cellular signaling pathways (7, 8). Whereas some of the initial data supporting this hypothesis was derived from cell fractionation methods that may be less specific than originally thought (9–11), more recent studies have demonstrated interactions between signaling molecules and caveolin using a variety of methods including immunoprecipitation, immunofluorescence microscopy, immunogold electron microscopy, and in vitro binding. These studies reveal that many proteins involved in mitogenic signaling cascades, including the epidermal growth factor, platelet-derived growth factor, insulin and Neu (c-ErbB2) receptors, c-Src, Fyn, Erk-2, and Ras, associate with caveolin (12–19). Similarly, various G protein-coupled signaling components including receptors (β2-adrenergic, m2-muscarinic, B2-bradykinin, cholecystokinin, ET-1/endothelin, calcium-sensing and angiotensin II receptors), G proteins (Gαi, Gαo, and Gαq) and various downstream effector molecules (adenyl cyclase, PKCα, Y-ε, and -ζ, and endothelial and neuronal nitric-oxide synthase) have been shown to interact with caveolin suggesting a potential role of caveolae in regulating such pathways (20–31). Since GRKs also play an important role in regulating G protein-coupled signaling pathways, we examined the primary sequence of the GRKs for consensus caveolin binding motifs (32). Indeed, GRK2 and -3 were found to contain a C-terminal, pleckstrin homology (PH) domain-localized consensus caveolin binding motif. Here, we investigate the interaction of GRKs with caveolin both in vitro and in intact cells, and we demonstrate a previously unappreciated mode of regulation for these kinases.
REGULATION OF GRKs BY CAVEOLIN

MATERIALS—Hemagglutinin (HA)-specific polyclonal antibody was from Babco. GRK1-, GRK5-, and caveolin-1-specific polyclonal antibodies were from Santa Cruz Biotechnology, and a caveolin-1-specific monoclonal antibody (2297) was from Transduction Laboratories, Inc. AP-2 α-subunit- and clathrin-specific antibodies were generously provided by Dr. J. H. Keen. Transferrin receptor-specific antibody was from Chemicon International. Maltose-binding protein (MBP) vector, pMAL, and amylose resin were from New England Biolabs. Most other reagents were from sources previously described (4).

Protein Expression and Purification—GRK2 and GRK5 were overexpressed in and purified from Sf9 insect cells (33, 34), and purified GRK1 was generously provided by Drs. J. Pitcher and R. J. Lefkowitz. Purified GST, GST-caveolin-1, GST-GRK2, and GST-GRK5 fusion proteins, and urea-stripped rod outer segment segments were prepared as described previously (27, 35, 36). Sf9 expressed and purified G9γ, and GRK2 PH domain were generously provided by Dr. S. P. Kennedy. An MBP-caveolin-1 fusion construct containing caveolin residues 1–101 (MBP-caveolin1–101) was generated by polymerase chain reaction amplification of base pairs 1–303 of the caveolin-1 cDNA. The product was then subcloned into the pMal vector in-frame with the upstream MBP via EcoRI and HindIII restriction sites in the polylinker region. Purified MBP and MBP-caveolin-1 fusion proteins were generated as described previously (37).

Cell Electrophoresis and Immunoblotting—SDS-PAGE was performed using standard methods (38). Following electrophoresis, proteins were electroblotted onto nitrocellulose. Immunoblotting was performed using caveolin-1, GRK1-, GRK2-, GRK5-, AP-2, clathrin, transferrin receptor-, Gβγ-, and GST-specific primary antibodies, horse-radish peroxidase-conjugated secondary antibody (1:2000 dilution), and detection following the manufacturer’s guidelines. GST-Caveolin and Fusion Binding Assay—Five μg of purified GST or GST-caveolin-1 fusion proteins containing either the N-terminal residues 1–61 (GST-caveolin1–61) or membrane proximal residues 61–101 (GST-caveolin61–101) immobilized on glutathione-agarose beads were incubated with 2 μg of purified GRK1, GRK2, GRK2 PH domain (GRK2 residues 553–670), GRK5 or G9γ in 100 μl of binding buffer (20 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 100 μM EDTA, 100 mM NaCl, and 0.2% Triton X-100) at 30 °C for 60 min. The samples were chilled on ice for 5 min, and the beads were then pelleted for 10 s, washed three times with 400 μl of binding buffer, and boiled with SDS sample buffer. Samples were subjected to 10% SDS-PAGE and immunoblotting with GRK- or Gβγ-specific antibodies.

MBP-Caveolin Fusion Binding Assay—One μg of purified MBP or MBP-caveolin1–101 immobilized on amylose resin was incubated with 200 μg of purified GRK2 or soluble GST-GRK2 (residues 1–147, 1–122, 1–88, 63–101, 180–184, 84–184, 94–184, 185–467, or 468–689), or GST-GRK5 (residues 1–200, 1–98, 1–39, 20–49, or 489–590) fusion proteins in 100 μl of binding buffer (20 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 100 mM NaCl, and 0.2% Triton X-100) at 30 °C for 60 min. Washing, elution, and analysis were identical to GST-caveolin binding experiments with the exception that GST fusion proteins were immunoblotted with a GST-specific antibody.

Cell Culture and Transfection—COS-1, A431 and NIH-3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (or 10% bovine calf serum for NIH-3T3 cells), 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate at 37 °C in a humidified atmosphere containing 5% CO2. COS-1 cells grown to confluence, and caveolin-rich fractions were generated by following the manufacturer’s guidelines.

RESULTS AND DISCUSSION

In Vitro Interaction of Caveolin and GRKs—Since many of the proteins involved in G protein-coupled receptor signaling associate with caveoleae and/or caveolin, we analyzed the primary sequence of GRK2 and found that a consensus caveolin binding motif (dsXXXdXXXdX, where d is any amino acid) is found within the C-terminal PH domain (residues 81–101) previously implicated in scaffolding domain (residues 81–101) previously implicated in caveolin interaction with other signaling molecules such as the epidermal growth factor receptor, c-Src, PKC, and endothelial nitric-oxide synthase (12, 17, 28, 30). Importantly, there was minimal binding to GST alone (data not shown) or GST-caveolin1–61 (Fig. 1). Moreover, GRK2 binding to GST-caveolin1–101 was modestly enhanced at higher ionic strength (data not shown), consistent with the binding being primarily mediated by hydrophobic interactions. Given the localization of the identified consensus caveolin binding sequence to the PH domain, we also analyzed the direct binding of purified GRK2 PH domain and observed significant and specific binding to GST-caveolin1–101 (data not shown). Interestingly, GRK1 and GRK5, which lack a PH domain, also displayed specific binding to GST-caveolin1–101 (although a low level of binding to GST-caveolin1–61 was also observed for GRK5) (Fig. 1). To further test the binding specificity, similar experiments were performed in the presence of 0.1% Triton X-100 in order to minimize the role of non-specific hydrophilic interactions. Whereas the overall extent of binding was slightly reduced for all of the GRKs tested, GRK1, GRK2, GRK5, and the GRK2-PH domain, retained specific binding to GST-caveolin1–101 (data not shown). To demonstrate further the specificity of GRK/caveolin interactions, we tested the binding of purified Gβγ to the GST-caveolin-1 constructs. Despite
in agreement with the observed binding of purified GRK2 PH domain to GST-caveolin 1–101, whereas a C-terminal construct (GST-GRK51–39) failed to bind (Fig. 2). To map further the N-terminal caveolin-binding site, GST-GRK51–98, GST-GRK51–39, and GST-GRK520–49 were analyzed. Although GST-GRK51–98 retained binding to MBP-caveolin1–101, GST-GRK51–39 and GST-GRK520–49 failed to bind (Fig. 2) suggesting that the critical caveolin binding region in GRK5 lies between residues 49 and 98, a region that overlaps the N-terminal caveolin binding region identified in GRK2. These data suggest that the N-terminal region, including GRK2 residues 63–71 (LXXXDXXDXX) (Fig. 2C), is important for the conserved GRK/caveolin binding characteristics. The initial study that proposed caveolin binding motifs (DXAAXXXDXXDXXDXXDXX) or DXAAXXXDXXDXXDXX has been identified in Go subunits, the Goα subunit has substitutions of valine and leucine for two of these aromatic residues (32). Despite this substitution, Goα has been shown to co-immunoprecipitate with caveolin (22). Thus, it seems possible that caveolin binding requirements may be broader than initially described. Finally, although the functional significance for the existence of two caveolin binding regions in GRK2 (residues 63–71 and the PH domain) remains to be elucidated, it is of interest that the PH domain-localized motif overlaps with a region that includes phospholipid-binding determinants (39).

**Interaction of GRK2 with Caveolin in Intact Cells**—Two approaches were used to ascertain whether GRK2 and caveolin associate in intact cells. The first employed a widely used extraction and fractionation method that enables the separation of caveolae from other cellular organelles (18). For these studies, A431 cells were lysed in a detergent-free sodium carbonate buffer, fractionated on a discontinuous sucrose gradient, and then analyzed for endogenous caveolin and GRK2 by SDS-PAGE and immunoblotting. As expected, the 5/35% sucrose interface (fraction 3) contained the bulk of the cellular caveolin, whereas most of the cellular protein was found in fractions 8 and 9 (Fig. 3A). Analysis of GRK2 revealed that while it was primarily present in fractions 8 and 9, a significant portion also co-fractionated with caveolin (Fig. 3A). A similar GRK2/caveolin co-fractionation was observed in NIH-3T3 cells (data not shown). Importantly, a number of molecules known to associate either peripherally (AP-2 or clathrin) or integrally (transferrin receptor) with a variety of cellular membranes including the plasma membrane, endosomes, the endoplasmic reticulum, and the Golgi (40, 41) were found to be almost exclusively restricted to fractions 8 and 9 (Fig. 3A). Thus, these
data confirm that this fractionation method generates a specific enrichment of certain cellular membranes including caveolae while excluding most others. However, several recent studies have further characterized the membranes and associated proteins found in caveolin-rich fractions generated from these or similar methods and demonstrated the presence of non-caveolin-containing membranes (9–11). Thus, although our data are consistent with caveolar localization of GRK2 in A431 and NIH-3T3 cells, we cannot rule out the possibility that GRK2 might be associated with one of these other compartments.

In order to demonstrate directly a GRK2/caveolin association in cells, we used an immunoprecipitation approach. In these studies, COS-1 cells co-expressing GRK2 and caveolin-1 were lysed in the presence of detergent (1% Triton X-100 and 60 mM octyl glucoside) in order to solubilize caveolin. Cleared lysates were then incubated with either HA-, GRK2-, or caveolin-specific polyclonal antibodies and then precipitated by addition of protein A-agarose and centrifugation. Bound proteins were eluted with SDS buffer and subjected to SDS-PAGE and immunoblotting using GRK2- and caveolin-specific monoclonal antibodies. As expected, GRK2 and caveolin were effectively precipitated by their respective specific antibodies whereas HA antibodies failed to precipitate detectable GRK2 or caveolin. Importantly, when GRK2 immunoprecipitations were probed with monoclonal antibodies for caveolin-1, a significant amount of caveolin was detected (~3% of the total) (Fig. 3B). Similarly, caveolin immunoprecipitations probed with a monoclonal GRK2 antibody revealed a significant amount of associated GRK2 (~6% of the total) (Fig. 3B). To demonstrate further the specificity of this method, immunoprecipitation using a transferrin receptor-specific monoclonal antibody was performed. This antibody significantly precipitated transferrin receptor but failed to precipitate detectable levels of either caveolin or GRK2 (data not shown). Thus, these data demonstrate that a portion of the cellular GRK2 was specifically associated with caveolin in these cells and remained associated during the subsequent solubilization in 1% Triton X-100 and 60 mM octyl glucoside and immunoprecipitation.

Taken together, the co-fractionation and co-immunoprecipitation of GRK2 and caveolin suggest that GRK/caveolin interactions are likely to occur in vivo. It is important to note that many studies have assessed GRK2/3 partitioning to membrane and cytosolic cell fractions using a variety of methods. In these studies GRK2/3 was found to be primarily cytoplasmic with only a small fraction (5–15%) associated with the membrane (2, 42, 43). Thus, the fraction of the total cellular GRK2 associated

FIG. 2. In vitro binding of soluble GST-GRK fusion proteins to a MBP-caveolin fusion protein. A, 1 μg of MBP-caveolin1–101 was incubated with 200 ng of GST-GRK2 or GST-GRK5 fusion proteins as described under "Experimental Procedures." Samples were subjected to SDS-PAGE and immunoblotting using a GST-specific antibody. Representative blots demonstrating binding of the indicated GST-GRK2 or GST-GRK5 fusion protein to MBP-caveolin1–101 are shown. Standards (Std) containing 10% of the total of either GST-GRK2 or GST-GRK5 are shown on the left. B, a schematic representation of GRK domains included in the binding assay is shown with binding activity summarized as either + (++) or no binding (−) on the right. C, alignment of GRK sequences in the region of GRK2 residues 63–71 mapped to contain caveolin-binding determinants. Conserved aromatic and hydrophobic residues that may be important for caveolin binding are shaded.
with the caveolin-rich fraction (Fig. 3A) and with caveolin
immunoprecipitates (Fig. 3B) likely represents a large portion
of the membrane-associated GRK2. Therefore, membrane-as-
associated events, such as GRK-mediated receptor phosphoryla-
tion, may be significantly affected by GRK/caveolin interaction.

Functional Significance of GRK/Caveolin Interaction—In
order to explore the potential functional significance of the
GRK/caveolin interaction, we considered that GRK activity
may be regulated by the caveolin scaffolding domain in a fash-
ion similar to the previously demonstrated inhibition of the
epidermal growth factor receptor (12), c-Src (17), PKC (28),
adenyl cyclase (29), and endothelial nitric-oxide synthase
(30). In order to test this hypothesis, GRK2 phosphorylation
of the receptor substrate rhodopsin was examined in the presence
of either scaffolding domain peptides from caveolin-1, -2, or -3
or a control peptide, caveolin-153–81 (Fig. 4A). In addition,
because the scaffolding domain peptides are very hydrophobic,
we also included a partially scrambled caveolin-1 scaffolding
domain peptide in these studies (30) (Fig. 4A). Since the amino
acid composition of this peptide is 100% identical to the caveo-
lin-1 scaffolding domain peptide, it should enable discrimina-
tion between sequence-specific binding and nonspecific hydro-
phobic or ionic interactions. In these studies caveolin-1 and
caveolin-3 scaffolding domain peptides effectively inhibited
GRK2 activity in a dose-dependent manner with IC50 values of
-0.4 and -0.8 μM, respectively (Fig. 4B). In contrast, the
caveolin-2 scaffolding domain and caveolin-153–81 control pep-
tides had no effect on GRK2 phosphorylation of rhodopsin.
Analysis of the other GRKs revealed that GRK1 was also ef-
effectively inhibited by caveolin-1 and -3 scaffolding domain pep-
tides with IC50 values of -2.7 and -1.8 μM, respectively, as
was GRK5 with IC50 values of -3.0 and -2.5 μM, respectively
(data not shown). Similarly, GRK1 and GRK5 activity were
unaffected by the caveolin-2 scaffolding domain or caveolin-
153–81 control peptides. Importantly, for all of the GRKs tested
the partially scrambled caveolin-1 peptide was found to be
unaffected by the caveolin-2 scaffolding domain or caveolin-
153–81 control peptides. Importantly, for all of the GRKs tested
the partially scrambled caveolin-1 peptide was found to be
dramatically less effective at inhibiting receptor phosphoryla-
tion (Fig. 4B). Given that this peptide is 100% identical in
sequence, it is not surprising that this peptide is partially inhibitory albeit with significantly reduced potency. Taken together, these results provide good
evidence that the observed inhibition of GRK-mediated recep-
tor phosphorylation by caveolin scaffolding domain peptides is
due to sequence-specific GRK/caveolin binding. It is worth not-
ing that the average IC50 for caveolin peptide inhibition of
GRK2-mediated rhodopsin phosphorylation is >4-fold lower
than the average IC50 for GRK1/GRK5 inhibition. This differ-
eence may be related to the presence of the second caveolin
binding region in GRK2 located within the PH domain. In fact,
the caveolin binding motif present in the PH domain overlaps
a region previously suggested to be important for binding acidic
phospholipids (39). Thus, it is possible that caveolin could com-
pete with phospholipid binding resulting in additional inhibi-
tion of GRK2-mediated rhodopsin phosphorylation.

In principle, the inhibition of GRKs might be attributed to (i)
inhibition of the catalytic domain through either direct or
allosteric mechanisms or (ii) inhibition of receptor binding to
critical determinants outside of the catalytic domain. In order
to discriminate between these two possibilities, we tested the
effects of the caveolin scaffolding domain peptides on GRK2
phosphorylation of the peptide substrate RRREEEEEEEAAA. In
these experiments, all caveolin peptides demonstrated a simi-
lar profile for inhibition of peptide phosphorylation as with the
receptor, with the caveolin-1 and -3 scaffolding domain pepe-
tides exhibiting inhibition with IC50 values of ~0.9 and ~0.8
μM, respectively. The partially scrambled caveolin-1 peptide
inhibited GRK2 only at higher concentrations, with an IC50
greater than 10-fold higher (~12 μM) than that of the wild type
peptide/GRK1-1 peptide (Fig. 4C). These data suggest that the
inhibitory properties of the caveolin peptides are specific and
likely to be exerted on the catalytic domain rather than by perturbing
receptor/GRK interactions outside of the catalytic domain.

In order to discriminate between competitive (direct) and
noncompetitive (allosteric) modes of inhibition, kinetic analysis
was performed by phosphorylating different concentrations of
peptide substrate in the presence of 0, 1, or 3 μM caveolin-1
scaffolding domain peptide. An Eadie-Scatchard plot of these
data reveal a profile suggestive of a predominantly noncompet-
tive/allosteric mode of inhibition (Fig. 4D). Similarly, a double-
reciprocal plot of the peptide phosphorylation data yielded K
values of 12.8 and 13.3 mM peptide substrate for reactions
performed in the presence of 0 and 3 μM caveolin peptide,
respectively, whereas the Vmax changed from 19.6 to 8.1 nmol
P/min/mg in the presence of 0 and 3 μM caveolin peptide,
respectively (data not shown). Thus, it appears that an ally-
steric mode of inhibition is likely to be the predominant mech-
anism for caveolin peptide-mediated inhibition of GRKs. This is
consistent with the mapping of caveolin-binding determinants
Our results suggest that GRKs may be subject to some level of tonic inhibition in cells that express caveolin-1 or -3. This potential functional role may be of particular importance in cardiac myocytes under the pathological condition of congestive heart failure. Sustained β-agonist stimulation of the mouse heart results in characteristic features of congestive heart failure including left ventricular hypertrophy and decreased β-adrenergic receptor signaling (45–47). Such chronic stimulation also results in a 2–3-fold increase in left ventricular GRK2 and GRK5 levels, an occurrence that may contribute to the pathological decrease in β-adrenergic receptor signaling in congestive heart failure (46, 47). Interestingly, a recent study demonstrated an ∼2-fold decrease in myocyte caveolin expression in hearts from mice subjected to sustained β-agonist stimulation (48). Thus, it seems plausible that in the failing cardiac myocyte a concomitant increase in GRK and decrease in caveolin expression could have combined effects on GRK activity and consequently the β-agonist responsiveness of the myocyte.

In summary, the above data identify a novel interaction between GRKs and caveolin that results in potent inhibition of GRK activity. These interactions are suggested to occur in vivo by the co-fractionation and co-immunoprecipitation of GRK2 and caveolin in cells. Although the overall cellular role of this interaction remains to be elucidated, one possibility is that caveolin serves to suppress basal GRK activity. Another possibility is that localization of GRKs in caveolae may direct a distinct set of protein/protein interactions thereby regulating GRK specificity. Indeed, the cellular localization and consequently the substrate specificity of protein kinase A is regulated in such a fashion via its interaction with a family of scaffolding proteins (49, 50). Thus, future investigation into this area should include assessment of novel caveolae-dependent protein interactions for GRKs, as well as further elucidation of the physiological role of GRK/caveolin interaction.

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FIG. 4. Inhibition of GRK activity by caveolin scaffolding domain peptides. A, sequences of the caveolin-1, -2, and -3 (CAV-1, -2, and -3) scaffolding domain peptides are aligned along with a partially scrambled caveolin-1 scaffolding domain peptide (CAV-1Δ). Residues conserved with caveolin-1 are shaded. The sequence of a control peptide, caveolin-1X, (CAV-1X), is also shown. B, inhibition of GRK-mediated rhodopsin phosphorylation. Peptide phosphorylation reactions were performed at 30 °C for 5 min and contained 30 nM GRK2 and 400 nM rhodopsin in the presence of 0–12.5 μM caveolin scaffolding domain peptides, CAV-1 (○), -2 (●) or -3 (□) or control peptides CAV-1X (■) and CAV-1Δ (■), as described under “Experimental Procedures.” The samples were subjected to SDS-PAGE, and the extent of rhodopsin phosphorylation was determined by excising and counting the 32P-labeled bands. Rhodopsin phosphorylation, expressed as a fraction of control (i.e. vehicle only), is plotted for various concentrations of peptide. C, inhibition of GRK2-mediated peptide phosphorylation. Peptide phosphorylation reactions were performed at 30 °C for 60 min and contained 50 nM GRK2, 1 μM RRREEEESAAA, and 0–12.5 μM caveolin peptide as stated above in B. Reactions were blotted on to P81 paper, washed in phosphoric acid, and counted to quantitate extent of phosphorylation. GRK2-mediated phosphorylation was taken as counts/min above control samples lacking GRK2, and these data were plotted as described above. D, kinetic analysis of GRK2-mediated peptide phosphorylation. Peptide phosphorylation reactions were performed at 30 °C for 20 min and contained 50 nM GRK2, 0.1–10 μM RRREEEESAAA and 0 (○), 1 (□), or 3 (△) μM caveolin-1 scaffolding domain peptide. Reactions were stopped as described above, and the resulting data were used to generate an Eadie-Scatchard plot. All values are mean ± S.E. from three separate experiments.

to sites outside of the catalytic domain. The fact that the GRK1,-2, and -5 are all inhibited by caveolin suggests that the identified conserved N-terminal site (Fig. 2) is likely to be most important for mediating this inhibition. However, caveolin binding to the PH domain of GRK2 may additionally contribute to its allosteric regulation. Indeed, binding of acidic phospholipids to this domain of GRK2 has been shown to mediate allosteric effects on GRK2 activity (44).
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