Recent data have demonstrated that caveolin, a major structural protein of caveolae, negatively regulates signaling molecules localized to caveolae. The interaction of caveolin with several caveolae-associated signaling proteins is mediated by the binding of the scaffolding region of caveolin to a hydrophobic amino acid-containing region within the regulated proteins. The presence of a similar motif within the insulin receptor kinase prompted us to investigate the caveolar localization and regulation of the insulin receptor by caveolin. We found that overexpression of caveolin-3 augmented insulin-stimulated phosphorylation of insulin receptor substrate-1 in 293T cells but not the phosphorylation of insulin receptor. Peptides corresponding to the scaffolding domain of caveolin potently stimulated insulin receptor kinase activity toward insulin receptor substrate-1 or a Src-derived peptide in vitro and in a caveolin subtype-dependent fashion. Peptides from caveolin-2 exhibited no effect, whereas caveolin-1 and -3 stimulated activity 10- and 17-fold, respectively. Peptides which increased insulin receptor kinase activity did so without affecting insulin receptor auto-phosphorylation. Furthermore, the insulin receptor bound to immobilized caveolin peptides, and this binding was inhibited in the presence of free caveolin-3 peptides. Thus, we have identified a novel mechanism by which the insulin receptor is bound and activated by specific caveolin subtypes. Furthermore, these data define a new role for caveolin as an activator of signaling.

Caveolae are cell-surface invaginations distinct from coated pits. Early studies suggested that caveolae play a role in molecular transport across the membrane; caveolae are capable of moving tracers, introduced into the blood space, across the endothelial cell by transcytosis (1). Other, more recent studies have proposed that caveolae function as subcellular compartments to concentrate molecules and provide them with a site for molecular interaction at the plasma membrane (2, 3).

A variety of signaling molecules are found to be enriched in caveolae and/or recruited to caveolae upon activation, such as various receptors, protein kinase C, G proteins (heterotrimeric and small), and non-receptor and receptor tyrosine kinases (4–11).

Caveolin, a principal component of the coat component of caveolae, is a major phosphoprotein in v-Src-transformed cells (12). Although the significance of the diversity of this subtype and their functional differences remain unknown, there are three known mammalian members with distinct tissue distributions (7, 13–15), as well as caveolin subtypes from invertebrates (Caenorhabditis elegans), suggesting the existence of an evolutionarily conserved multi-gene family (7, 13, 14, 16). Recent studies have suggested regulatory as well as structural functions for caveolin; caveolin may directly regulate numerous signaling proteins in caveolae. Different groups have demonstrated inhibition of G proteins, Src family kinases, nicotinic acid synthase, epidermal growth factor receptor, and protein kinase C by a short stretch of the membrane proximal region of the cytosolic amino-terminal caveolin domain (or the caveolin scaffolding domain), leading to the suggestion that the biological function of caveolin is to suppress cellular signaling (5, 9–15, 21). The specificity of caveolin interaction to these molecules has been confirmed by identifying a common amino acid sequence motif (ψXεXXXψ or ψXXXεXXX; where ψ is an aromatic residue), which is contained in many of these regulatory molecules and in the insulin receptor (IR) as well (19).

Like the cell-surface receptors for many peptide growth factors and hormones, the IR contains an endogenous tyrosine kinase within its cytoplasmic domain. This kinase activity is required for transmission of downstream insulin signals, such as energy storage and cell proliferation (22, 23). The IR tyrosine kinase is partially activated by insulin binding and fully activated following so-called “auto-phosphorylation” of the regulatory loop region within the kinase domain. Activation of the IR kinase allows the tyrosine phosphorylation of intracellular substrates of the IR, i.e. the insulin receptor substrate (IRS) proteins, such as IRS-1 (22, 23).

Histological data from adipocytes demonstrated that radiiodinated insulin bound to membrane microinvaginations distinct from α2-macroglobulin-methylamine binding coated pits (24), implying that IRSs are accumulated in caveolae (25). Recent studies have also demonstrated that insulin regulates the distribution of caveolin (26) and that insulin stimulates phosphorylation of caveolin protein (27, 28).

In this study, we show that the IR is indeed enriched in caveolae by co-fractionation. We further investigated whether...
there was a productive regulatory interaction between the IR and caveolin. We demonstrate that the scaffolding domain of caveolin interacts with the IR, leading to an increase in IR kinase activity.

EXPERIMENTAL PROCEDURES

Materials—Antisera against IRS-1, the COOH terminus of the IR, and phosphotyrosine have been previously described (29). Antisera against caveolin-1 and -3 were purchased from Transduction Laboratories (Lexington, KY). [γ-32P]ATP was obtained from NEN Life Science Products. Most other reagents were obtained from Sigma. A 13-mer peptide (RRLIEDAEYARG) was from Life Technologies, Inc. (30). Caveolin peptides were synthesized as described previously (19). Briefly, the six peptides used most frequently in this study were as follows: a caveolin-1 non-scaffolding domain peptide (NDPQKHLNDDVKIDFEDVIAEPEGTHSF, caveolin-1 amino acid residues 53–81); the caveolin-1 scaffolding domain peptide (DGHWKASPTTFTVTKYW-FYR, amino acid residues 82–101); the caveolin-2 scaffolding domain peptide (DKVWCHSLFEISKKYMYK, amino acid residues 54–73); the caveolin-3 scaffolding domain peptide (DGWVRAYSSTFTSVSKYW-CYR, amino acid residues 55–74); two peptides derived from IR (GLLPVRWMAFESLKDGVF, amino acid residues 1157–1174); (TTSS-DMWSFGQVVIWEITS, amino acid residues 1175–1192, containing the caveolin binding motif). The peptides were synthesized by Research Genetics (Huntsville, AL) or Biosynthesis (Lewisville, TX). Peptides were dissolved in 100% Me2SO and diluted to a final concentration of 1.25–10 μM at the time of assay. The final concentration of Me2SO was kept constant among assay tubes at 0.5 or 1%. 

Sucrose Gradient Centrifugation—Caveolin-enriched membrane domains were purified from cultured CHO cells overexpressing the human IR and IRS-1 (31) using a previously optimized method (7, 32). Cells were resuspended in 2 ml of 500 mM sodium carbonate (pH 11) and homogenized with 12 strokes of a Dounce homogenizer, three 10-s bursts of a Polytron tissue grinder, and three 20-s bursts of a sonicator. The sucrone concentration in cell extracts was adjusted to 45% by the addition of 2 ml of 90% sucrose prepared in MBS (25 mM Mes, pH 6.5; 1.25 M NaCl), and the extracts were placed at the bottom of an ultracentrifuge tube. A 5–35% discontinuous sucrose gradient was formed above (4 ml of 35/4 ml of 5% sucrose, both prepared in MBS containing 250 mM sodium carbonate) and centrifuged at 39,000 rpm for 16 h at 4 °C in a Sorvall TH 641 rotor. A light-scattering band was confined to 4 °C in a Sorvall TH 641 rotor. A light-scattering band was confined to the gradient fractions (1 ml each) were collected. Gradient fractions were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (6 or 10%) and overlaid onto the cells, followed by incubation at 37 °C in 5% CO2. After 45 min the LipofectAMINE/cDNA solution was diluted with 4.8 ml of DMEM supplemented with 5% fetal bovine serum and antibiotics. In our preliminary experiments, the efficiency of transfection as estimated using a plasmid harboring β-galactosidase gene was over 50%.2

Immunoprecipitation was performed after incubation for 48 h. Cells were treated with insulin (10 nm) in the media for each time. Cells were washed twice with phosphate-buffered saline and homogenized in a buffer containing 1% Nonidet P-40, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1 mM NaPPi, 100 mM NaF, 1 mM sodium orthovanadate, and protease inhibitors. Insoluble material was removed by centrifugation at 10,000 × g for 10 min, and IR antibodies were added overnight at 4 °C. Immunocomplexes were collected on protein A-Sepharose and washed three times with ice-cold 1% Nonidet P-40, 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1 mM NaPPi, 100 mM NaF, 1 mM sodium orthovanadate, and 0.1% SDS. Immunoblotting was performed using an antibody against phosphotyrosine or IR, and their expression was quantitated with the use of a computer densitometer (Molecular Dynamics, Sunnyvale, CA).

Overexpression and Purification of BIRK and IRS-1—The cytosolic domain of IRK was overexpressed using the baculovirus expression system (BIRK) as described previously (kindly provided by Dr. J. Ahn) (34, 35). Cells were homogenized in a buffer containing 50 mM Tris-HCl (pH 7.4), 1 mM DTT, 250 mM sucrose, 0.5 mM EDTA, and protease inhibitors by sonication three times (10 s) at 4 °C, followed by centrifugation at 100,000 × g for 40 min at 4 °C. The resulting supernatant was used as the crude cytosolic source of BIRK. The purified BIRK was obtained from the cytosolic fraction through three rounds of chromatography (Fast Q-Sepharose, phenyl-Sepharose, and Superose-12 columns) as described previously (34, 35).

IRS-1 was similarly overexpressed in insect cells and purified through gel filtration chromatography on S300 HR as described previously (29). The purified IRS-1 was used as a substrate of BIRK.

In Vitro IR Kinase Assay—Either the cytosolic fraction of insect cells (approximately 2.5 μg/tube, which contained 2.5–5.0 pmol BIRK) or the purified BIRK (0.38 μg/tube or 7.9 pmol BIRK) was used. Phosphorylation reactions were carried out in a 40-μl buffer solution containing 50 mM Tris-HCl (pH 7.4), 4 mM MnCl2, 1 mM DTT, 100 μM [γ-32P]ATP (approximately 1,000 cpm/pmol ATP), 0.1% Triton X-100, 450 mM NaCl, 20 μM sodium orthovanadate, 1.0 mg/ml bovine serum albumin, and 0.5 or 1% Me2SO. The purified IRS-1 (1.5 μg/tube or 8.3 pmol) or an Src substrate peptide (500 μg/tube) was used as substrate. Assays were performed at 23 °C for 20 min and were stopped by adding 40 μl of 2× Laemmli buffer solution containing 100 mM DTT, 50 mM EDTA, and 50 mM EGTA. After boiling the reaction mixture for 5 min, the samples were applied to SDS-PAGE and exposed to autoradiography.

To separate the Src peptide, a 28% polyacrylamide gel containing 6 × urea was used. Phosphorylation of the substrate was quantitated with the use of a computer densitometer (Molecular Dynamics, Sunnyvale, CA).

Peptide Binding Assay—Caveolin peptides were immobilized on Affi-Gel 10 (Bio-Rad). Briefly, after washing the resin with 5 bed volumes of isopropl alcohol and Me2SO, the resin was incubated with 2 μM caveolin peptide overnight at room temperature. The coupling of peptides to the resin was confirmed by monitoring the recovery of peptides remaining in the supernatant. After 12 h, approximately 50% of the incubated peptide was bound to the resin. Any unreacted sites were blocked by the addition of an excess amount of ethanolamine. After extensive washing of the peptide-bound resin, the resin and the cytosolic solution overexpressing BIRK (approximately 0.125 mg/ml) were incubated for 10 h at 4 °C in a buffer containing 20 mM Tris-HCl (pH 7.4), 0.5% Triton X-100, 150 mM NaCl, 0.5 mg/ml bovine serum albumin, and protease inhibitors. The resin was washed extensively four times with an excess amount of the buffer, followed by the addition of Laemmli buffer and boiling for 5 min. After centrifugation, the supernatant was applied to SDS-PAGE, followed by immuno blotting. When BIRK binding to the resin was competed by free peptides, the cytosolic solution overexpressing BIRK (0.125 mg/ml) was preincubated with free peptides (50 μM) before adding to the caveolin peptide-bound resin. Incubation, washing, and elution procedures were as described above.

Peptides derived from IR were similarly immobilized on Affi-Gel 10. Briefly, the resin was incubated with 2 μM insulin receptor peptide overnight at room temperature. Membrane protein overexpressing caveolin-3 (approximately 0.15 mg/ml) was incubated for 10 h at 4 °C in

Fig. 1. Subcellular distribution of IRS-1, IR, and caveolin in CHO cells. CHO cells stable overexpressing the IR and IRS-1 were homogenized, followed by subcellular fractionation using a 5–35% discontinuous sucrose gradient method. From the top of each gradient, a total of 13 fractions was collected and subjected to SDS-PAGE. Immunoblotting was performed using antibodies raised against IRS-1 (top, IRS-1), IR (middle, IRK), and caveolin (bottom, Cav 1). Molecular weight markers are indicated on the left.
a buffer containing 50 mM Tris-HCl (pH 7.4), 0.5% Nonidet P-40, 150 mM NaCl, 1 mM DTT, and protease inhibitors. Washing and elution procedures were as described above. The samples were applied to SDS-PAGE, followed by immunoblotting.

RESULTS

IR Are Accumulated in Caveolin-enriched Fraction—Since an early histological study demonstrated that cell-surface insulin binding activity concentrated in non-coated microvaginations (24), we were interested in determining whether caveolin and the IR co-localize. We thus determined the location of the IR and caveolae in cells using a sucrose gradient method that has been commonly used to purify caveolar fractions from culture cells (7, 32).

Caveolin was abundantly expressed in CHO cells. CHO cells stably transfected with the IR and IRS-1 (31) were homogenized and fractionated by sucrose gradient centrifugation, followed by immunoblotting to examine the distribution of the IR, caveolin, and IRS-1 (Fig. 1). Caveolin, our caveolar marker,
was found in fractions 4–5, and the IR was similarly distributed. IRS-1, in contrast, was predominantly in fractions 9–12. Thus, our findings suggested that IR are present in plasmalemmal caveolae although the sucrose gradient method as well as any other currently available methods may not purify caveolae to homogeneity.

Overexpression of Caveolin Augments Insulin Signaling—Recent studies have demonstrated that caveolin subtypes are not merely structural proteins in caveolae but also interact with and regulate important signaling molecules in caveolae (5, 15–21, 36) and that caveolin-3 and -1 have generally higher regulatory activity than caveolin-2. We therefore examined the effect of caveolin-3 on IR signaling in cultured cells.

In order to have the greatest chance of observing an effect of caveolin-3 overexpression on IR function, we chose cells that express low levels of endogenous caveolin-1 and -3; HEK293T cells were derived from HEK293 cells by introducing the SV40 large T antigen, and like many oncogenically transformed cells (37), they expressed less caveolin than wild type parental HEK293 cells (data not shown). We assessed the effect of caveolin on IR activity by comparing insulin-dependent tyrosine phosphorylation of the endogenous IR substrate, IRS-1, in 293T cells and 293T cells overexpressing caveolin-3. When the HEK293T cells were stimulated with insulin, the phosphorylation of endogenous IRS-1 was increased in a time-dependent manner as detected by immunoblotting using an anti-phosphotyrosine antibody (Fig. 2A). In HEK293T cells overexpressing caveolin-3, the degree of insulin-stimulated tyrosine phosphorylation of IRS-1 was approximately 2 times greater than that in mock-transfected control cells (Fig. 2B). Insulin induced autophosphorylation of IR time-dependently; however, there was no augmentation in the degree of autophosphorylation in cells overexpressing caveolin-3. The content of IRS-1 and IR was not altered by overexpressing caveolin-3 (Fig. 2, A and C). Endogenous IGF-1 receptors were not detectable in HEK293T cells (data not shown).

These results demonstrate that caveolin-3 enhances insulin-stimulated tyrosine phosphorylation of IRS-1, suggesting that caveolin can activate as well as inhibit signaling protein function (5, 15–21, 36). It is not clear, however, whether this effect is directly through the IR or whether it is mediated indirectly by other insulin-stimulated tyrosine kinases in caveolae.

Caveolin Scaffolding Domain Peptides Stimulate IR Kinase Activity—In order to determine whether caveolin directly stimulates the IR tyrosine kinase, we examined its effect on the IR in vitro. We initially attempted to examine the effect of purified caveolin, but purified caveolin aggregated at high concentrations (38, 39). We thus used a short stretch of the membrane proximal region of the cytosolic amino-terminal caveolin domain (or the caveolin scaffolding domain). By using this peptide, different groups have demonstrated the caveolin-mediated inhibition of G proteins, Src kinases, and nitric oxide synthase (5, 15–20). We overexpressed the cytosolic kinase portion of human IR in insect cells (BIRK) as described previously (34, 35), and we examined the effect of the caveolin scaffolding domain peptides on the ability of BIRK to phosphorylate purified IRS-1 protein in vitro.

As shown in Fig. 3A, caveolin-1 and -3 scaffolding domain peptides increased BIRK activity significantly in a dose-dependent manner. The caveolin peptides from different lots or the peptides provided by different manufacturers showed similar effects. In contrast, the caveolin-2 scaffolding domain peptide, as well as a caveolin-1 non-scaffolding domain peptide (data not shown), had no effect on BIRK activity at any concentration. The degree of stimulation appeared to be greater for caveolin-3 (~17-fold) than for caveolin-1 (~10-fold). Auto-phosphorylation of BIRK was not altered in the presence of any of the above peptides. Similar results were obtained when immunoblotting using an anti-phosphotyrosine antibody was conducted (Fig. 3B).

The above study demonstrated that caveolin peptides stimulated BIRK. With these initial findings, we started investigating the mechanism of caveolin-mediated activation of BIRK.

BIRK Stimulation Assay with Src Peptides—We then examined the substrate specificity of the caveolin-mediated potentiation of BIRK activity because caveolin peptides may modify IRS-1 (not BIRK) to increase the degree of its IRS-1 phosphorylation. We examined the effect of caveolin peptides using an Src-derived peptide, a peptide substrate commonly used in BIRK assays (30). As shown in Fig. 4, caveolin peptides also stimulated IR kinase activity using this substrate. The caveolin-1 and -3 scaffolding domain peptides potentiated BIRK activity in a dose-dependent manner (Fig. 4). The degree of potentiation of BIRK activity with an Src-derived peptide was less than that with IRS-1; however, this may be due to differences in the geometries and/or accessibilities of the substrates for the enzyme active site. Thus, caveolin stimulated BIRK activity regardless of the substrate species.

The Caveolin-3 Peptide Stimulates Purified BIRK—We also tested whether caveolin peptides stimulated BIRK directly; caveolin peptides may increase BIRK activity indirectly.
through activating another factor(s) contained in the cell extract. We examined the effect of the caveolin-3 scaffolding domain peptide on BIRK, which was purified from insect cells to yield a single major band on SDS-PAGE with Coomassie staining (Fig. 5A). As shown in Fig. 5B, the caveolin-3 peptide stimulated the purified BIRK activity in a dose-dependent manner, suggesting that the stimulation of the caveolin peptide with BIRK was direct and independent of the other factors.

**Mutational Analysis of Caveolin-mediated Stimulatory Activity**—We also examined various deletion mutants of caveolin-1 and -3 peptides. The caveolin-1 scaffolding domain peptide was sequentially deleted from the amino terminus to produce 3 shorter peptides (20-mer, amino acid residues 82–101; 16-mer, amino acid residues 86–101; 14-mer, amino acid residues 88–101; 12-mer, amino acid residues 90–101). Similarly, the caveolin-3 scaffolding domain peptide was sequentially deleted (20-mer, amino acid residues 55–74; 17-mer, amino acid residues 58–74; 14-mer, amino acid residues 61–74; 11-mer, amino acid residues 64–74). BIRK overexpressed in H5 cells and IRS-1 was incubated in the presence of 10 μM of each peptide for assay.

As shown in Fig. 6, a 16-mer caveolin-1 peptide stimulated BIRK activity to a similar degree as a 20-mer peptide, whereas the rest of the peptides (below 14-mer) were mostly ineffective. Similarly, a 17-mer caveolin-3 peptide stimulated BIRK activity to a similar degree as a 20-mer peptide, but the rest of the peptides did not, suggesting that there is a length requirement for the stimulation of BIRK activity by these caveolin peptides.

**Caveolin Physically Interacts with Insulin Receptor Kinase**—Finally, to confirm the interaction of caveolin peptides with BIRK, we examined whether caveolin peptides directly bound to BIRK. The caveolin-3 scaffolding domain peptide was immobilized on Affi-Gel 10, followed by incubation with BIRK. After extensive washing, the bound BIRK was eluted from the resin. As shown in Fig. 7A, BIRK bound to the immobilized caveolin-3 scaffolding domain peptide or the caveolin-1 scaffolding domain peptide, but not to Affi-Gel alone nor to the caveolin-1 non-scaffolding domain peptide. These data suggest that BIRK directly bound to the caveolin peptides that stimulate BIRK activity. We also examined whether binding of BIRK to the immobilized caveolin peptide was competed by free caveolin peptide. BIRK was preincubated with free caveolin-3 scaffolding domain peptide before adding to the caveolin-3-immobilized resin. The binding of BIRK was significantly reduced by this competition (Fig. 7B).

We also examined the importance of the caveolin-binding motif within IR. We made a 18-mer peptide derived from the region containing the caveolin-binding motif within the IR kinase domain (TTSSDMWSFGVTVWEITS, amino acid residues 1175–1192). This peptide was immobilized on Affi-Gel 10, followed by incubation with the membrane protein overexpressing caveolin-3. After extensive washing, the bound caveolin-3 protein was eluted from the resin. As shown in Fig. 7C, caveolin-3 bound to this peptide with the motif but not to Affi-Gel alone nor to another IR peptide derived from its vicinity but lacking the motif (amino acid residues 1157–1174) (Fig. 7C).

Thus, caveolin most likely interacts with IR through the caveolin-binding motif that exists in IR. These data suggest that the interaction between caveolin peptide and insulin kinase domain was direct and specific.

**DISCUSSION**

Taken together, our data show that the IR interacts with caveolin and binds to the scaffolding region of specific subtypes


**FIG. 6.** Effect of mutated caveolin peptides on IR kinase activity. The caveolin-1 scaffolding domain peptide (A, 20-mer, amino acid residues 82–101) and the caveolin-3 scaffolding domain peptide (B, 20-mer, amino acid residues 55–74) were sequentially deleted from the amino terminus to generate three shorter peptides (A, Cav 1, caveolin-1, 16-mer, amino acid residues 86–101; 14-mer, 88–101; 12-mer, amino acid residues 90–101) (B, Cav 3, caveolin-3, 17-mer, amino acid residues 58–74; 14-mer, amino acid residues 61–74; 11-mer, amino acid residues 64–74). BIRK was incubated in the absence (Control) and presence of these peptides as well as [32P]ATP and IRS-1, followed by SDS-PAGE and autoradiography.

of caveolin (caveolin-3, and -1, but not caveolin-2). This insulin receptor/caveolin binding is direct and serves to increase the activity of the IR kinase independently of receptor autophosphorylation. Thus, caveolin appears to target the IR kinase to a specific subcellular location (caveolae) and stimulate the IR kinase activity toward substrates in caveolae. Since this effect is subtype-specific and caveolin subtypes have different tissue distributions, it is tempting to speculate that this is a mechanism for altering the function of the IR in a cell type-specific manner.

The result of the IR/caveolin interaction is functionally different from the interaction between caveolin and other signaling molecules, as caveolin inhibits the signaling function of these other molecules (5, 15–21, 36). The role of each caveolin subtype in regulating insulin signaling, however, was very similar to that previously reported with other kinase molecules (9, 20); both caveolin-1 and -3 regulated the function, whereas caveolin-2 had no effects on kinase activity. The same caveolin subtypes therefore increase the activity of the IR kinase while decreasing the activity of other signaling molecules. It is teleologically unclear why caveolin would increase IR kinase activity while decreasing the activity of other signaling molecules. It is teleologically unclear why caveolin would increase IR kinase activity while decreasing the activity of other signaling molecules. Expression of caveolin is known to change with cellular differentiation. When fibroblasts differentiate into adipocytes, caveolin expression increases with an increase in IR signaling (40). In contrast, other growth hormone signaling such as platelet-derived growth factor is decreased (41). Caveolin might play a role in cellular differentiation by coordinating the balance of different hormones and growth factor signaling. Furthermore, caveolin-3 is abundant in skeletal muscle, which strongly depends on insulin for energy metabolism. It is known that phosphofructokinase-M, a key regulatory enzyme in the glycolytic pathway in muscles, binds caveolin-3 and that this binding is stabilized in the presence of phosphofructokinase-M activators (42). Thus, caveolin may play an role in regulating energy metabolism in skeletal muscles as well.

Although we do not know the exact molecular mechanism by which caveolin stimulates the activity of the IR tyrosine kinase, it is clear that this is due to the direct binding of the receptor by the scaffolding domains of caveolins-3 and -1. The site of binding within IR is most likely within the region containing the caveolin-binding motif (TTSSDMWSFGVVIWEITS, amino acid residues 1175–1192). The IR kinase is activated by insulin binding and further activated by tyrosine phosphorylation of the kinase regulatory domain (22, 23). It is also known that the kinase regulatory domain is a protein loop which sits over the active site of the kinase in the inactive state; autophosphorylation of this loop acts to move it away from the kinase domain, allowing substrate access to the site and kinase activity. Given the proximity of the putative caveolin-binding site to the regulatory region, and remembering that caveolin binding increases activity but not autophosphorylation, it is tempting to speculate that caveolin stabilizes the activated conformation of the regulatory region of the receptor in an activated state.

Caveolin may selectively regulate the function of signaling molecules accumulated in caveolae by either increasing or suppressing their enzymatic activity. Here we have provided further evidence that caveolin is regulatory as well as structural and that it can enhance, as well as inhibit, the activity of cellular signaling molecules.

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