Identification of Peptide and Protein Ligands for the Caveolin-scaffolding Domain

IMPLICATIONS FOR THE INTERACTION OF CAVEOLIN WITH CAVEOLAE-ASSOCIATED PROTEINS*

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Caveolin, a 21–24-kDa integral membrane protein, is a principal component of caveola membranes. We have suggested that caveolin functions as a scaffolding protein to organize and concentrate certain caveolin-interacting proteins within caveola membranes. In this regard, caveolin co-purifies with a variety of lipid-modified signaling molecules, including G-proteins, Src-like kinases, Ha-Ras, and eNOS. Using several independent approaches, it has been shown that a 20-mer amino acid membrane proximal region of the cytosolic amino-terminal domain of caveolin is sufficient to mediate these interactions. For example, this domain interacts with G-protein α subunits and Src-like kinases and can functionally suppress their activity. This caveolin-derived protein domain has been termed the caveolin-scaffolding domain. However, it remains unknown how the caveolin-scaffolding domain recognizes these molecules.

Here, we have used the caveolin-scaffolding domain as a receptor to select random peptide ligands from phage display libraries. These caveolin-selected peptide ligands are rich in aromatic amino acids and have a characteristic spacing in many cases. A known caveolin-interacting protein, Gia2α, was used as a ligand to further investigate the nature of this interaction. Gia2α and other G-protein α subunits contain a single region that generally resembles the sequences derived from phage display. We show that this short peptide sequence derived from Gia2α interacts directly with the caveolin-scaffolding domain and competitively inhibits the interaction of the caveolin-scaffolding domain with the appropriate region of Gia2α. This interaction is strictly dependent on the presence of aromatic residues within the peptide ligand, as replacement of these residues with alanine or glycine prevents their interaction with the caveolin-scaffolding domain. In addition, we have used this interaction to define which residues within the caveolin-scaffolding domain are critical for recognizing these peptide and protein ligands. Also, we find that the scaffolding domains of caveolins 1 and 3 both recognize the same peptide ligands, whereas the corresponding domain within caveolin-2 fails to recognize these ligands under the same conditions. These results serve to further demonstrate the specificity of this interaction. The implications of our current findings are discussed regarding other caveolin- and caveola-associated proteins.

Caveolae are plasma membrane-attached vesicular organelles that have a characteristic diameter in the range of 50–100 nm (1, 2). Caveolae are present in most cell types but are especially abundant in adipocytes, endothelial cells, fibroblasts, and smooth muscle cells (3). In adipocytes and smooth muscle cells, they represent up to 20% of the total plasma membrane surface area. Endothelial cells contain ~5,000–10,000 caveolae/cell. Although they were originally implicated in cellular transport processes (4), recent evidence suggests that they may participate in signal transduction-related events (5–11).

Caveolin, a 21–24-kDa protein, is a principal integral membrane component of caveola membranes in vitro (12, 13). Using either Triton X-100-based methods or detergent-free methods, caveolin co-purifies with certain lipid-modified signaling molecules (such as G-proteins, Src family tyrosine kinases, and Ha-Ras) (5, 6, 7, 9, 10, 14–16). In addition, caveolin was first identified as a major v-Src substrate that undergoes tyrosine phosphorylation in Rous sarcoma virus-transformed cells (17). Based on these and other observations, we have proposed the “caveolae signaling hypothesis,” which states that caveolar localization of certain signaling molecules could provide a compartmental basis for their actions and explain cross-talk between signaling pathways (18–20).

Several independent lines of evidence suggest that caveolin may function as a scaffolding protein within caveola membranes: (i) both the amino- and carboxyl-terminal domains of caveolin remain entirely cytoplasmic and are therefore accessible for cytoplasmic protein-protein interactions (21); (ii) caveolin forms high molecular mass homo-oligomers of ~350 kDa (22, 23) that have the capacity to interact with specific lipids (cholesterol and glycosphingolipids; Refs. 24 and 25) and lipid-modified signaling molecules (G-proteins, Src family kinases, and Ha-Ras; Refs. 16 and 24–29); (iii) these caveolin homo-oligomers can self-assemble to form caveola-like structures in vitro (22, 25); and (iv) recombinant overexpression of caveolin in caveolin-negative cells (lymphocytes and Sf 21 insect cells) is sufficient to drive the formation of caveola-sized...
proteins (G-proteins, Ha-Ras, and Src family kinases), we have previously termed this protein domain the caveolin-scaffolding domain (29).

Here, we have used the caveolin-scaffolding domain and one of its protein ligands (a G-protein α subunit, Gαs) to explore the possible protein sequence requirements that underlie this molecular recognition event. As a first step, a fusion protein carrying the caveolin-scaffolding domain was used to select random peptide ligands from phage display libraries. Many of these peptide ligands share two properties with a particular region of the G-protein: (i) a preponderance of aromatic amino acids in a short stretch; and (ii) a characteristic spacing between these aromatic residues. We show that this region of Gαs interacts directly with the caveolin-scaffolding domain, and a peptide encoding this G-protein domain competitively inhibits the binding of the appropriate region of Gαs to the caveolin-scaffolding domain.

**MATERIALS AND METHODS**

**Phage Display Library Selection**—The 15-mer library and bacterial strains were from Dr. George Smith (University of Missouri, Columbia, MO), whereas the decapeptide (10-mer) library was constructed as described elsewhere (32). The GST-caveolin-1-(61–101) fusion protein was purified and immobilized on glutathione-agarose beads (Sigma) as described previously (26). The incubation was performed using 6 × 10^10 transforming units of phage in presence of 50 μl of beads in TNET buffer (10 mM Tris pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.05% Tween 20) at 4 °C under agitation for 16 h. Beads were then washed with 20 ml of TNET before acidic elution with 100 μl of 50 mM glycine, pH 2.2. The eluate was neutralized with 0.1 volume of 1 M Tris, pH 10.0, and used to infect *Escherichia coli* K91-kan cells for amplification. Purified phage were prepared as described elsewhere (33). Four rounds of selection were performed. Colonies from the last titering plate were picked to inoculate 2 ml of Luria broth/tretracycline medium. The culture was grown overnight, and phage DNA was prepared by polyethylene glycol precipitation of the culture supernatant, phenol-chloroform extraction and ethanol precipitation (33). The region corresponding to the selected peptide was sequenced using a Sequenase II kit from U.S. Biochemical Corp. An oligonucleotide specific for the fuse5 vector downstream of the cloning site was used for sequencing (5'-GCCGTGATCCATTCCA-CAGACAA-3').

**Phage Binding Assays**—We used 96-well plates (Nunc Maxisorp) to perform all ELISAs. Phage from selected clones were prepared as described elsewhere (33). Purified phage in Tris-buffered saline (TBS) were used for coating the plate, and the incubation proceeded at 4 °C overnight. Plates were saturated for 1 h at room temperature with TBS/0.05% Tween 20 (TBST) containing 1% bovine serum albumin. Purified His-tagged Muc full-length caveolin-1 (1 μg/well) (25) or GST-caveolin-1-(61–101) fusion protein (1 μg/well) (26) was then added to TBST to the well for 2 h and washed several times with TBST. Monoclonal antibodies directed against the Muc epitope (Harvard Monoclonal Antibody Facility, Cambridge, MA) (1:400) or GST (Santa Cruz Biotechnology) (1:1000) in TBST were then added for 1 h at room temperature followed, after washes, by horseradish peroxidase anti-mouse IgG (Amersham Corp.) (1:2000). The reaction was revealed using 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate (6)] (Boehringer Mannheim), and absorbance was measured on a microplate reader at 410 nm. The opposite assay was also performed, in which purified full-length caveolin-1-Muc-HCl (1 μg/well) or GST-caveolin-1-(61–101) fusion proteins (1 μg/well) were coated onto the wells in 100 mM sodium bicarbonate, pH 8.5, and then incubated with phage. A biotinylated anti-Fc polyclonal antibody (Sigma) (1:1000) was used in addition to horseradish peroxidase-streptavidin (Zymed) (1:2000) and 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate (6)]. For the ELISA using peptides, 500 pmol/well was used for coating the wells in bicarbonate coating buffer. Peptide synthesis was performed by the Biopolymers Facility at the Massachusetts Institute of Technology (Cambridge, MA) and Research Genetics (Huntsville, AL).

**GST-Gαs Fusion Proteins**—Using the bovine Gαs cDNA (provided by Dr. Nukada) as a template, the polymerase chain reaction was performed to construct GST-Gαs-full length (1-355), GST-Gαs-B (120-238), and GST-Gαs-C (239-355). The products were subcloned into the EcoRI site of the pGEX-1T vector; resulting constructions were subjected to restriction analysis and sequencing via the Sanger method. Purification of GST-Gαs fusion proteins was essentially as described.

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1 The abbreviations used are: GST, glutathione S-transferase; ELISA, enzyme-linked immunosorbent assay; TBS, Tris-buffered saline; TBST, TBS/Tween 20; FL, full-length.
Sequence, occurrence, and aromatic residue content of peptide ligands selected using a GST fusion protein containing the caveolin-1-scaffolding domain

Single-stranded DNA was purified from phage clones after four rounds of selection for binding to the GST-caveolin-1-(61–101) fusion protein. Peptide sequences were deduced from the DNA sequencing of the corresponding region of the bacteriophage genome.

| Sequence                              | 10-mer |           | 15-mer |           |
|---------------------------------------|--------|-----------|--------|-----------|
|                                       | Occurrence | W, F, or Y content | Occurrence | W, F, or Y content |
| MWHWEKRKWV                            | 13     | 3         | RNVPFFNOVDVVWAF | 31     | 4         |
| KRNAEGLNDREV                          | 2      | 3         | RNAAAVVEGAFNYV | 3      | 3         |
| HRAAEVRMRBR                           | 2      | 3         | TEPWEGTERVNEG | 2      | 4         |
| MRRESCCEWE                            | 1      | 3         | TRWGEDSPFIRSPG | 1      | 2         |
| MRVEHENADE                            | 1      | 3         | PAVVirTEGSGWNY | 1      | 4         |
| VNASRALRQTVV                          | 1      | 3         | GGGGQERPFLYGMFFD | 1 | 5         |
| VMAHRAVTREN                           | 1      | 3         | CSSAYGTTRVLCA | 1      | 3         |
| MREERSSRBE                            | 1      | 3         | IGRHYHSTGSPS | 1      | 2         |
| RENTQGSHMLGL                          | 1      | 3         | ECHLELLECRVWGR | 1 | 3         |
| KNLQGSSLRGE                           | 1      | 3         | WSVRYDEVLVPSLPP | 1 | 4         |
| RDAVGHVCXL                            | 1      | 2         | SSGRDAEQQSDGOA | 1      | 3         |
|                                       |        |           |        |           |

**Caveolin, "Velcro" for Signal Transduction**

![Graphs of amino acid abundance](image)

**Fig. 2.** Relative abundance of the 20 different amino acids in 10- and 15-mer peptides displayed by their respective bacteriophage libraries, before and after selection for caveolin binding. A and C, single-stranded DNA was purified from 25 (10-mer, 11 different clones) and 55 (15-mer, 22 different clones) clones obtained after four rounds of selection. The sequences of the peptides displayed were deduced by DNA sequencing. The percentage of occurrence for each amino acid calculated as the number observed divided by the total number of residues in the peptides is shown. B and D, peptide sequence of 18 (10-mer) and 30 (15-mer) random bacteriophage clones from the unselected starting libraries, respectively. Calculations were made as described for A and C. Amino acids are grouped according to the number of codons that specify them. The frequency expected for each group is shown by the dotted line and assumes that all codons were used with equal efficiency.
Figure 3. Binding specificity of caveolin-selected phage clones and peptides. A, each of the caveolin-1 peptides was coated in the bottom of wells of an ELISA plate. The assay was performed as described under "Materials and Methods." A negative result was assumed for an absorbance level (at 410 nm) less than two times of that recorded for negative controls (i.e. caveolin peptide incubated without phage or peptide plus buffer alone). In this case, a positive result (+) indicates a level of absorbance more than 10 times of those with respective negative controls, nd, not determined. Each determination was performed in duplicate. B, left, relative binding of the most abundant 15-mer clone (phage or the corresponding biotinylated peptide, biotin-RNVPPIFNDVYWIAF) to the scaffolding domain of caveolin-1 (residues 82–101) and to the same region divided into two peptides. The assay was as described for A. Binding to the caveolin-scaffolding domain was arbitrarily set as 100%. Each determination was performed in duplicate. Right, scaffolding domains for caveolin-1 (residues 82–101), caveolin-2 (Cav-2, residues 54–73), and caveolin-3 (Cav-3, residues 55–74) were compared for their relative binding to the most abundant 15-mer clone (phage or the corresponding biotinylated peptide). Binding to the scaffolding domain of caveolin-1 was arbitrarily set at 100%. All results represent the means ± S.D. (bars) of triplicate determinations.

RESULTS

Identification of Peptide Ligands for the Caveolin-scaffolding Domain Using Phage Display—We used a GST-caveolin fusion protein that contains the caveolin-scaffolding domain as a receptor to randomly select peptide ligands from two different phage display libraries (Fig. 1). This GST-caveolin fusion protein contains caveolin residues 61–101 and has been previously shown to be functionally sufficient to interact with G-protein α subunits (including Gαs, c-Src, and Ha-Ras (26, 29). After four rounds of selection, caveolin-binding phage clones were subjected to DNA sequence analysis to reveal their peptide sequences, as shown in Table I. Caveolin binding clones from the 10-mer library were rich in tryptophan, and most exhibited a characteristic spacing conforming to the sequence WXXWXXXW. Random sequencing of the 10-mer library indicated that tryptophan was enriched ~3-fold in caveolin-selected clones (Fig. 2, A and B). In contrast, caveolin binding clones from the 15-mer library did not exhibit any characteristic spacing but were also enriched in aromatic amino acids. More specifically, tryptophan, phenylalanine, and tyrosine were enriched 3.8-, 1.5-, and 1.8-fold relative to the unselected library population (Fig. 2, B and C). Also, a single 15-mer clone (RNVPPIFNDVYWIAF) accounted for ~60% of all clones. An ELISA was developed to evaluate the interaction of these peptide ligands with various regions of caveolin. Nine caveolin-derived peptides that correspond to regions of the cytoplasmic amino-terminal domain of caveolin were used to as receptors to capture these ligands (Fig. 3A). All phage clones tested only interacted with the peptide that corresponds to the caveolin-scaffolding domain (residues 82–101; Fig. 3A) and with recombinant full-length caveolin-1 purified from E. coli (not shown). The clones tested include the three most abundant clones from the 10- and 15-mer libraries (see Table I) and the clone containing the sequence VWEWAVSRFN. Identical results were obtained when a biotinylated peptide corresponding to the sequence of the most abundant 15-mer phage clone was tested.

When the caveolin-scaffolding domain is divided into two halves (residues 84–92 and 93–101), we have previously shown that this inhibits its functional activity (26). Similarly, each of...
recent studies have shown that caveolin is the first member of a multigene family of related molecules (13, 27, 28, 35, 36); caveolin has been retermed caveolin-1. Thus, we next examined the interaction of these caveolin-selected phage clones with the scaffolding domains of caveolins 1–3. Fig. 3A shows that these peptide ligands only interact with the scaffolding domains of caveolins 1 and 3 but fail to interact with the homologous domain in caveolin-2. This suggests that the scaffolding domain of caveolin-2 has different protein sequence requirements for its interaction with other molecules and further demonstrates the selectivity of these interactions, as these peptide ligands were selected using the scaffolding domain of caveolin-1.

Interaction of G\textsubscript{2a}α-derived Proteins and Peptide Domains with the Caveolin-scaffolding Domain—By comparing the peptide ligands obtained for the caveolin-scaffolding domain with the protein sequences of a known class of caveolin-interacting proteins (G-protein α subunits), we deduced two possible caveolin-binding motifs: 8XΦ0XXXΦ and 8XΩXXΦXXΦ, where Φ represents any amino acid. A peptide (designated GP) was designed for use in binding studies, and its sequence corresponds to the one illustrated here for the G\textsubscript{2a}α subunit. B, schematic diagram summarizing the construction of different GST-G\textsubscript{2a}α fusion proteins used to examine interactions with caveolin (FL, B, and C).

To evaluate whether this region of G-protein α subunits can serve as a ligand for the caveolin-scaffolding domain, we constructed: (i) a synthetic peptide containing this G-protein region (THFTFKDLHFKMFDV), termed GP; and (ii) a variety of GST-G\textsubscript{2a}α fusion proteins, including full-length (FL), G\textsubscript{2a}α and two deletion mutants, termed B and C (Fig. 4B). Note that the GP region is contained within two of these GST-G\textsubscript{2a}α fusion proteins (FL and B).

Fig. 5A shows that both the FL and B G\textsubscript{2a}α fusion proteins interact preferentially with the caveolin-scaffolding domain, but little or no interaction was observed with an adjacent region of caveolin (residues 53–81). Also, the B region of G\textsubscript{2a}α was only recognized by the scaffolding domains of caveolins 1 and 3 but not by the scaffolding domain of caveolin-2 (Fig. 5B). This binding profile is exactly what we observed previously for peptide ligands selected using the scaffolding domain of caveolin-1 (See Fig. 3B), suggesting a similar mode of interaction.

Fig. 6A shows that the GP peptide interacts with the scaffolding domain of caveolins 1 and 3 and that this interaction is strictly dependent on the presence of aromatic residues within the GP peptide. When the phenylalanine residues of the GP peptide (THFTFKDLHFKMFDV) were changed to either alanine or glycine (THATFKDLHAKMADV and THTGFKLHLCGKMDV), binding of these peptides to the caveolin-scaffolding domain was almost completely abolished. This is consistent with the idea that aromatic residues play a key role in recognition by the caveolin-scaffolding domain. Also, it is important to note that the GP peptide was only recognized by the scaffolding domains of caveolins 1 and 3 but not by the scaffolding domain of caveolin-2 (Fig. 6A).

The GP peptide also competitively inhibits the binding of the...
The GP peptide interacts with the caveolin-scaffolding domain and competitively inhibits the binding of a GST-Gi₂\(_{B}\) fusion protein. A, the biotinylated GP peptide was evaluated for its ability to interact with the scaffolding domains of caveolins 1–3. The binding of two mutated GP peptides was also evaluated in parallel. In these mutant GP peptides, all four phenylalanine residues were changed to glycine (Phe → Gly) or alanine (Phe → Ala). Binding to an irrelevant region of caveolin-1 (residues 53–81) was included as a negative control. Note that the wild-type GP peptide interacts with the scaffolding domains of caveolins 1 and 3 but only weakly with the same region of caveolin-2. In contrast, the mutant GP peptides showed little or no interaction with any of the caveolin domains tested. B, affinity-purified GST-Gi₂\(_{B}\) fusion protein (120 ng) was allowed to interact with the scaffolding domain of caveolin-1 in the absence or presence competing peptide. Two different peptides containing caveolin binding motifs (the GP peptide and the 15-mer peptide) competitively inhibited the binding of the GST-Gi₂\(_{B}\) B fusion protein to caveolin-1. In contrast, two mutant GP peptides were unable to inhibit this interaction. All results are expressed as the means ± S.D. (bars) of triplicate determinations.

The B region of G\(_{12α}\) to the caveolin-scaffolding domain, and this occurs in a dose-dependent manner (Fig. 6B). In this regard, it is important to note that the B region contains the sequence that corresponds to the GP peptide. Virtually identical results were obtained using the peptide ligand selected from the 15-mer phage display library (RNVPPFFNDVYWIAF), indicating that this caveolin-selected peptide, the GP peptide, and G\(_{12α}\) are all recognized by the caveolin-scaffolding domain in a similar fashion. Importantly, mutated GP peptides lacking phenylalanine failed to show any competition for binding.

Fig. 7 shows that the GP peptide also recognizes the full-length intact caveolin-1 molecule (caveolin-1-Myc-H\(_{7}\)). However, as predicted, the GP peptide was not recognized by full-length caveolin-2 (caveolin-2-Myc-H\(_{7}\)) under identical conditions. These results provide an extraordinary demonstration of the specificity of this interaction given the close protein sequence homology between caveolins 1 and 2. Caveolin-2 is 58% similar and 38% identical to caveolin-1 (27).

As the protein sequence encoded by the GP peptide appears to serve as a ligand for the caveolin-scaffolding domain, we identified the location of this sequence within the known three-dimensional structure of G-protein \(\alpha\) subunits. The GP region lies directly between switch I and switch II regions and precisely defines the space between where switch I ends and switch II begins (Fig. 8). Interestingly, we have previously shown that a Gln → Leu mutation (located 5 amino acid residues downstream from the end of the GP region) prevents the interaction of G\(_{12α}\) with caveolin-1 (26). This mutation also locks the G-protein \(\alpha\) subunit in the GTP-ligated and -activated conformation.

**Mutational Analysis of the Caveolin-1-scaffolding Domain**—

Two different approaches were used to define critical residues within the caveolin-1-scaffolding domain that are required for binding peptide ligands. First, deletion mutagenesis of the 82–101 region indicated that a minimal length of 16 amino acids is required (residues 86–101) (Fig. 9A). Second, alanine-scanning mutagenesis was then performed using this minimal caveolin-1-scaffolding domain. Our results indicate that a central core of four amino acids (F\(_T\)V\(_T\)Y\(_T\)) is strictly required for interaction of the caveolin-1-scaffolding domain with both the GP peptide and the corresponding region of G\(_{12α}\) (Fig. 9B). This region is F\(_T\)V\(_T\)S in caveolin-3 and F\(_E\)I\(_S\) in caveolin-2. Thus, this small difference may explain why these peptide ligands (library peptides and GP peptide) and protein ligands (GST-G\(_{12α}\), fusion proteins) are recognized by the scaffolding domains of caveolins.
DISCUSSION

Here, we have identified peptide and protein ligands for the scaffolding domain of caveolin and characterized the sequence requirements of this reciprocal interaction. Several independent lines of evidence indicate that this interaction is extremely specific: (i) the identified peptide and protein ligands interacted only with the caveolin-scaffolding domain, but not with other regions of the caveolin protein; (ii) the interaction also occurred with the purified full-length caveolin molecule expressed as a polyhistidine-tagged protein; (iii) the interaction was sequencesspecific; mutation of critical phenylalanine residues within the ligand prevented the binding of these ligands to the caveolin-scaffolding domain; also, mutation of certain critical residues within the caveolin-scaffolding domain abrogated the binding of peptide and protein ligands; and (iv) these peptide and protein ligands bound selectively to the scaffolding domains of caveolins 1 and 3 but not to the scaffolding domain of caveolin-2. This is despite the fact that these domains within caveolins 1–3 are extremely homologous. Also, it is important to note that these ligands were identified using the scaffolding domain of caveolin-1, suggesting that other potential ligands may exist that selectively recognize the scaffolding domain of caveolin-2. These results are consistent with the previous observations that the scaffolding domains of caveolins 1 and 3 can both act as GDP dissociation inhibitors for heterotrimeric G-proteins, and both inhibit the autoactivation of Src family kinases (16, 26–29). In contrast, the scaffolding domain of caveolin-2 exhibits GTPase-activating activity toward heterotrimeric G-proteins and fails to affect the activity of Src family kinases (27, 29).

What are the possible implications of these interactions? Other modular protein domains (such as Src homology 2 and 3, WW, and PID) have been previously defined and their corresponding peptide and protein ligands identified using techniques that are similar or identical to the ones used here for the caveolin-scaffolding domain. Src homology 2 domains and PID domains both recognize phosphotyrosine and 3 or 4 surrounding residues; Src homology 3 domains recognize proline-rich sequences, especially with the consensus PXXP; and the WW domain recognizes proline-rich sequences such as PPRP (see Prosite data base for specific references). Protein kinases also recognize short peptide sequences to direct phosphorylation of specific peptide and protein substrates: protein kinase A (R-R-XX-(2)-[DE]); protein kinase C ([ST]-X-[RK]); casein kinase II ([ST]-X-(2)-[DE]); and tyrosine kinases ([RK]-X-(2,3)-[DE]-X-(2,3)-Y). This is also the case for other posttranslational modifications, including: N-glycosylation (N-X-[ST]); N-myristoylation (MG); dual acylation (MGC); and prenylation (C-aliphatic-aliphatic-X). Similarly, antibody binding depends on the recognition of specific epitopes that can be as small as 5–10 amino acids in length. Thus, many diverse cellular processes rely heavily on the recognition of short consensus peptide motifs. However, not all of these motifs are recognized, as this depends on whether a given motif is cytoplasmic or extracellular or is exposed on the surface of the protein; exposure on the surface of the protein may even be conformation-specific. Also, recognition may depend on the subcellular localization of the protein and the potential interacting partner, e.g. nuclear, cytoplasmic, plasma membrane, or Golgi-associated. These added criteria for interaction thus strictly modulate whether the interaction may or may not take place and greatly increase the specificity of these interactions. Of course, these same constraints would apply to the recognition of peptide and protein ligands by the caveolin-scaffolding domain.
A deletion mutants of the caveolin-1-scaffolding domain were constructed and tested for binding to the GST-Gia2 B fusion protein. Results are expressed relative to the binding observed for the complete caveolin-scaffolding domain (residues 82–101) as the means ± S.D. of three determinations. A minimal domain of 16 amino acids (residues 86–101) was required for recognition of the GST-Gia2 B fusion protein. Each residue within this minimal caveolin-1-scaffolding domain was systematically replaced one at a time with alanine and tested for interaction with the GST-Gia2 B fusion protein and the GP peptide. Results are expressed in percentages relative to the control nonmutated region as the means ± S.D. of three determinations.

An increasing number of reports suggest that many different classes of molecules are concentrated within caveolin-rich membrane domains. Thus, we searched the protein sequences of known caveolin- or caveoleae-associated proteins for aromatic-rich sequences that contain a specific spacing (φXφXXXφXXφ or φXXXφXXXφXXφ, where φ = Trp, Phe, or Tyr), as defined here using the caveolin-scaffolding domain. Most molecules reported to be associated with caveolin or caveoleae contain cytoplasmically accessible sequences that resemble those that we have defined here as peptide and protein ligands for the caveolin-scaffolding domain (summarized in Table II). Although it is not yet known whether all these molecules interact directly with caveolin, our studies provide a rational and systematic basis for investigating whether these protein sequences are indeed recognized as cytoplasmic ligands by the caveolin-scaffolding domain. This type of interaction could provide a simple mechanism for sequestration of a diverse group of molecules within caveolin-rich regions of the plasma membrane.

The caveolin-binding motif is reminiscent of another motif identified using the chaperone BiP for selection: Hy/WXHyXHyXHyXHy, where Hy is a large hydrophobic amino acid (most frequently Trp, Leu, or Phe) and X is any amino acid (37). In fact, the best peptides tested for binding to BiP contain one Phe and three Trp, which suggests that they could also bind to caveolin. However, for BiP, no single peptide sequence was enriched over others. This is in contrast to the present situation, in which strong enrichments were observed for particular caveolin-selected peptide sequences. The apparent similarity between BiP and caveolin in this matter may suggest a potential role for caveolin as a chaperone. Although to our knowledge no chaperone described thus far is an integral membrane protein, caveolin could fulfill the role of a “membrane-bound chaperone” by interacting with signaling molecules and maintaining them in an inactive conformation.

**REFERENCES**

1. Anderson, R. G. W., Kamen, B. A., Rothberg, K. G., and Lacey, S. W. (1992) Science 255, 410–411
2. Anderson, R. G. W. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 10909–10913
3. Barra, N. J. (1988) J. Cell Sci. 90, 341–348
4. Simionescu, N., Simionescu, M., and Palade, G. E. (1975) J. Cell Biol. 64, 586–607
5. Sargiacomo, M., Sudol, M., Tang, Z.-L., and Lisanti, M. P. (1993) J. Cell Biol. 122, 789–807
6. Lisanti, M. P., Scherer, P. E., Vidugiriene, J., Tang, Z.-L., Hermanski-Vanakta, A., Yu, Y.-H., Cook, R. F., and Sargiacomo, M. (1994) J. Cell Biol. 126, 111–126
7. Chang, W. J., Ying, Y., Rothberg, K., Hooper, N., Turner, A., Gambicl, H., De Gunzburg, J., Mumbey, S., Gilman, A., and Anderson, R. G. W. (1994) J. Cell Biol. 126, 127–138
8. Chun, M., Liyanage, U., Lisanti, M. P., and Lodish, H. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11728–11732
9. Robbins, S., Quintrell, N., and Bishop, J. M. (1995) J. Cell. Biochem. Suppl.
10. Shenoy-Scaria, A. M., Dietzen, D. J., Kwong, J., Link, D. C., and Lublin, D. M. (1994) *J. Cell Biol.* **126**, 353–363

11. Travis, J. (1993) *Science* **262**, 1208–1209

12. Rothberg, K. G., Heuser, J. E., Donzell, W. C., Ying, Y., Glenney, J. R., and Anderson, R. G. W. (1992) *Cell* **68**, 673–682

13. Glenney, J. R., and Soppet, D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 10517–10521

14. Scherer, P. E., Lisanti, M. P., Baldini, G., Sargiacomo, M., Corley-Mastick, C., and Lodish, H. F. (1994) *J. Cell Biol.* **127**, 1233–1243

15. Smart, E. J., Ying, Y., Mineo, C., and Anderson, R. G. W. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 10104–10108

16. Song, K. S., Li, S., Okamoto, T., Chun, M., Sargiacomo, M., and Lisanti, M. P. (1996) *J. Biol. Chem.* **271**, 9690–9697

17. Glenney, J. R., and Zokas, L. (1989) *J. Cell Biol.* **108**, 2401–2408

18. Lisanti, M. P., Scherer, P., Tang, Z.-L., and Sargiacomo, M. (1994) *Trends Cell Biol.* **4**, 231–235

19. Lisanti, M. P., Scherer, P. E., Tang, Z.-L., Kubler, E., Koleske, A. J., and Sargiacomo, M. S. (1995) *Semin. Dev. Biol.* **6**, 47–58

20. Dupree, P., Parton, R. G., Raposo, G., Kurzchalia, T. V., and Simons, K. (1993) *EMBO J.* **12**, 1597–1605

21. Monier, S., Parton, R. G., Vogel, F., Behlke, J., Henske, A., and Kurzchalia, T. (1995) *Mol. Biol. Cell* **6**, 911–927

22. Murata, M., Peranen, J., Schreiner, R., Weiland, F., Kurzchalia, T., and Simons, K. (1994) *Biochim Biophys Acta* **1206**, 10299–10303

23. Li, S., Okamoto, T., Scherer, P. E., Tang, Z.-L., Kubler, E., Song, K. S., Sanders, M. C., and Lisanti, M. P. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 9407–9411

24. Mo, K., Parton, R. G., Raposo, G., Kurzchalia, T. V., and Simons, K. (1995) *Mol. Biol. Cell* **6**, 231–235

25. Fujimoto, T. (1993) *J. Cell Biol.* **120**, 1147–1157

26. Hermouet, S., Merendino Jr., J. J., Gutkind, J. S., and Spiegel, A. M. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 10455–10459

27. Lyons, J., Landis, C. A., Harsh, G., Vallar, L., Grunewald, K., Feichtinger, H., Duh, Q. Y., Clark, O. H., Kawasaki, E., Bousse, H. R., and McCormick, F. (1990) *Science* **249**, 655–659

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