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The Binding Site for the βγ Subunits of Heterotrimeric G Proteins on the β-Adrenergic Receptor Kinase*

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The βγ subunits of heterotrimeric G proteins play important roles in regulating receptor-stimulated signal transduction processes. Recently appreciated among these is their role in the signaling events that lead to the phosphorylation and subsequent desensitization of muscarinic cholinergic (Haga, K., and Haga, T. (1992) *J. Biol. Chem.* 267, 2222–2227) and β-adrenergic (Pitcher, J. A., Inglese, J., Higgins, J. B., Arriza, J. L., Casey, P. J., Kim, C., Benovic, J. L., Kwatra, M. M., Caron, M. G., and Lefkowitz, R. J. (1992) *Science* 257, 1264–1267) receptors. βγ mediates the membrane targeting of the β-adrenergic receptor kinase (βARK), in response to receptor activation, through a specific βARK-βγ interaction. This process utilizes the membrane-anchoring properties of the isoprenylated γ subunit of βγ.

In the present study, we have employed three distinct approaches to identify the region within the carboxyl terminus of βARK which binds βγ and thereby results in membrane translocation. We studied the ability of βγ to enhance the enzymatic activity of a series of truncated mutants of bovine βARK1, the ability of glutathione *S*-transferase fusion proteins containing various lengths of the carboxyl terminus of βARK to bind βγ subunits, and the ability of synthetic peptides comprised of βARK sequences to inhibit βγ activation of βARK1. We find that the minimal βγ binding domain of βARK is localized to a 125-amino acid residue stretch, the distal end of which is located 19 residues from the carboxyl terminus. A single 28-mer peptide (Trp^{643} to Ser^{670}) derived from this sequence effectively inhibited βγ activation of βARK1, with an IC_{50} of 76 μM. The identification of this "βγ binding domain" on βARK and the development of peptide inhibitors provide important tools for the study of G protein-coupled receptor desensitization, as well as for the investigation of βγ activation of other G protein-effector systems.

The heterotrimeric guanine nucleotide-binding proteins (G proteins),[1] comprised of α, β, and γ subunits mediate cellular signal transduction in response to a wide range of extracellular stimuli (1–3). For several years interest has focused on the GTP-binding α subunits and their activation of numerous effector molecules which include enzymes and ion channels. The tightly associated βγ subunits were initially believed to play only a supporting role, in which they served merely as regulators of "activated" α subunit levels. The structural and mechanistic diversity initially discovered within the α subunit gene family seemed to support this concept that the GTPase-containing α subunit was the active component of G proteins (1–3). In recent years, however, several β and γ isoforms have been isolated (3) which display specificity not only in their interaction with one another (4, 5) but in coupling specific receptors to a common effector (6). These data, along with several recent reports of βγ modulation of effector enzymes and ion channels, have contributed to an increasing realization of the prominent involvement of βγ in several transmembrane signaling systems.

The list of G protein-coupled effectors which appear to be modulated by βγ subunits is rapidly expanding and now includes certain isoforms of enzymes such as phospholipase C (7) and adenylate cyclase (8, 9). βγ has also been shown to modulate potassium channels coupled to cardiac muscarinic receptors (10), the pheromone-induced mating response in *Saccharomyces cerevisae* (11) and possibly phospholipase A_2 (12). The effects of βγ upon these effectors include a conditional (*i.e.* along with α_s) stimulation of type II adenylate cyclase (8, 9). Moreover, in very recent work, Kleuss *et al.* (6) have shown that different β subunits are responsible for coupling specific pituitary calcium currents to somatostatin or muscarinic cholinergic receptors.

The actions of βγ described above all pertain to effector molecules which are responsible for the cellular responses seen as a result of G protein-coupled receptor activation. Recently, a novel action of βγ in G protein-coupled receptor signal transduction has been uncovered where the actions of βγ facilitate the phosphorylation of muscarinic cholinergic (13, 14) and β-adrenergic receptors (15, 16). The specific phosphorylation of activated receptors is associated with a diminished responsiveness to additional agonist, a process generally referred to as desensitization (17, 18). The G protein-coupled receptor kinases are the enzymes responsible for this agonist-dependent receptor modification. This kinase family includes the β-adrenergic receptor kinase isozymes (βARK1 (19) and βARK2 (20)), rhodopsin kinase (RK) (21), and likely other members not yet isolated. We recently reported that βγ specifically mediates the translocation of cytosolic βARK1 to the plasma membrane where it phosphorylates activated receptor substrate (15). This translocation is mediated via direct binding of the membrane-anchored βγ to βARK. The γ subunit of non-retinal G proteins is modified at its carboxyl terminus by the geranylgeranyl isoprenoid

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[1] The abbreviations used are: G protein, guanine nucleotide regulatory protein; βARK, β-adrenergic receptor kinase; RK, rhodopsin kinase; ROS, rod outer segments; PAGE, polyacrylamide gel electrophoresis; GST, glutathione *S*-transferase; PBS, phosphate-buffered saline. Where applicable three letter and single letter amino acid codings are utilized.

moiety (22, 23) which functions to anchor the βARK-βγ complex at the receptor-membrane interface (15, 16).

Similarly in the phototransduction system, the specific membrane translocation of another G protein-coupled receptor kinase, RK, is also isoprenoid-dependent (16). The carboxyl terminus of RK is modified covalently with a farnesyl isoprenoid moiety (24), and this lipid modification is directly responsible for the light-dependent "docking" of the kinase to rod outer segment (ROS) membranes where it then phosphorylates light-activated rhodopsin (16). The translocation and activation of both RK and βARK are thus dependent on isoprenylation but the non-prenylated state of βARK necessitates the formation of a protein complex with βγ to acquire an isoprenoid moiety. To date, virtually no information is available concerning the molecular basis for the interaction between βγ and any of its disparate effectors. Accordingly, the present study was undertaken to identify the specific region(s) on βARK which directly interact with and bind to the βγ subunits.

EXPERIMENTAL PROCEDURES

Construction of Truncated βARK and Fusion Protein cDNAs—The cloned bovine βARK1 coding region (19) inserted as a *Hin*dIII-*Bam*HI fragment into the mammalian expression vector pBC12BI (25) was used as the wild-type βARK1 template for all molecular manipulations. Truncated βARK1 cDNAs were constructed via standard polymerase chain reaction techniques which we have described previously for mutant βARK and RK constructs (16). 3′-Primers encoding new truncated carboxyl termini were paired with 5′-primers corresponding to βARK1 cDNA sequence at either the *Rsr*II (nucleotide 1617) or *Xho*I (nucleotide 1780) sites which represent the two unique restriction endonuclease sites utilized for the splicing of the amplified mutant cassettes. Truncated constructs were verified by dideoxy sequencing (26) using T7 Polymerase (Pharmacia LKB Biotechnology Inc.).

For *Escherichia coli* expression of fusion proteins, the GST gene fusion vector pGEX-2T (Pharmacia) was used to make cDNA constructs in which various lengths of βARK carboxyl-terminal regions were ligated in-frame with the 3′-end of the coding region for GST. Polymerase chain reaction-amplified cassettes using specific 5′- and 3′-primers of different carboxyl-terminal regions of βARK1 or βARK2 were ligated as *Bam*HI-*Eco*RI fragments, and clones utilized in these experiments were verified by dideoxy sequencing as described above.

Expression of cDNA Constructs in COS 7 Cells and E. coli—Truncated βARK1 cDNAs described above were transfected into COS 7 cells using a standard DEAE-dextran procedure described previously (25). Transiently expressed kinases were metabolically labeled with [^{35}S]methionine (Du Pont-New England Nuclear). The cells were then harvested, lysed, and the soluble cell extracts containing the mutant kinases were quantitated via immunoprecipitation and Western blot analysis using βARK antibodies as described (16). The COS 7 cell supernatants stored on ice at 4 °C served as the source of kinases used in all subsequent assays.

Fusion protein constructs were introduced into the *E. coli* strain NM522 and induced with isopropyl-1-thio-β-D-galactopyranoside to produce overexpression of the GST/βARK carboxyl-terminal fusion proteins. The fusion proteins were purified by affinity chromatography on glutathione-Sepharose 4B (Pharmacia) essentially as described (27). The fusion proteins were stored as stock solutions at a concentration of 1 mg/ml in phosphate-buffered saline (PBS), 1 mM dithiothreitol, and 1 mM EDTA. The integrity of the purified fusion proteins was checked by SDS-PAGE and Coomassie Blue staining prior to use in the assays described below.

Phosphorylation Assays—The activity of the transiently expressed kinases in COS 7 cell extracts was determined by their ability to phosphorylate rhodopsin present in ROS membranes. Dark-adapted bovine retinas were obtained from the Hormel Co., and rhodopsin-enriched ROS membranes were prepared by sucrose gradient centrifugation and urea-stripping as described previously (28). Phosphorylation of the purified ROS membranes was carried out with COS 7 supernatants containing wild-type βARK1 or truncated βARK1 mutants under identical conditions as we have described (16). In the current study, reactions were bleached with light for 5 min prior to quenching with SDS loading dye. The samples then underwent SDS-PAGE and the level of phosphorylation, indicated by specific $^{32}P_i$ incorporation, was determined by scintillation counting of the dried gel slices containing rhodopsin and subtraction of the nonspecific $^{32}P_i$ incorporation produced by COS 7 supernatants transfected only with vector (15, 16). For the determination of βγ activation of kinase activity, various concentrations of purified brain βγ were incubated in the phosphorylation mixture. The βγ subunits were purified from bovine brain as described by Casey *et al.* (29). The concentration of βγ required to produce half-maximal βARK1 activation in wild-type and truncated kinases was determined by using a rectangular hyperbolic function (Sigmaplot software).

Detection of βγ Binding to GST-βARK Fusion Proteins—The binding of βγ to carboxyl-terminal fusion proteins of βARK was accomplished essentially as we have described previously (15). Briefly, the βARK carboxyl-terminal GST-fusion proteins described above or GST (negative control) were diluted in 50 μl of PBS containing 0.01% Lubrol to a final concentration of 500 nM fusion protein. To this solution was added bovine brain βγ to a final concentration of 50 nM. After incubation for 20 min at 4 °C, 20 μl of a 50% slurry of glutathione-Sepharose 4B in PBS was added and the incubation was continued on ice for 20 min. The Sepharose beads containing the bound fusion protein-βγ complex were washed three times with 400-μl aliquots of PBS containing 0.01% Lubrol. Retained proteins were removed from the Sepharose beads with SDS-PAGE sample buffer, subjected to SDS-PAGE on 12% acrylamide gels, and transferred to nitrocellulose. Antibodies to β (Du Pont-New England Nuclear) were used at a dilution of 1:1000, and blots were developed with goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (Bio-Rad).

Inhibition of βγ Activation Using Synthetic Peptides and Fusion Proteins—Peptides corresponding to specific βARK1 sequences were synthesized as their amino-terminal-acylated and carboxyl-terminal-amidated forms on an ABI model 430A peptide synthesizer using Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) chemistry. All peptides were purified by reverse phase high performance liquid chromatography on a dynamax C-18 300 Å column using conditions similar to those described previously (30). IC_{50} values of synthetic peptides and GST-βARK fusion proteins for the βγ activation of βARK1 activity were obtained by performing ROS phosphorylation assays as described above except that varying concentrations of peptides and fusion proteins were incubated with fixed βARK1 (20 nM) and βγ (100 nM) concentrations. IC_{50} values were determined using a four parameter logistic function (Sigmaplot software).

RESULTS AND DISCUSSION

βγ Activation of Truncated βARK1 Mutants Expressed in COS 7 Cells—As depicted in Fig. 1*a*, the primary sequence of βARK1 can be visualized as containing three domains of approximately equal size, a carboxyl domain, an amino-terminal domain, and the centrally located catalytic domain. cDNAs encoding several βARK1 carboxyl-terminal truncations were constructed and expressed in COS 7 cells (Fig. 1, *a* and *b*). These sequential truncations allowed us to map the distal end of the region, which is responsible for activation by βγ, within the large carboxyl terminus of βARK1, where βγ is known to bind (15). The truncated βARK1 constructs were tested for their ability to specifically phosphorylate rhodopsin present in purified bovine ROS membranes which serves as a substrate for βARK1 (Fig. 1*c*). Kinase activity was assessed in the absence and presence of increasing concentrations of purified brain βγ subunits. Truncating 19 amino acids from the carboxyl terminus of βARK1 (Fig. 1*a*, *construct 2*) does not result in any significant loss of βγ-activation of the kinase (Fig. 1*c*). Further removal of an additional 9 amino acids (Fig. 1*a*, *construct 4*) results in a complete loss of activation of this truncated kinase by βγ (Fig. 1*c*). The basal activities (defined as ROS membrane phosphorylation in the absence of βγ) of wild-type βARK1, construct 2, and construct 4 were similar (Fig. 1*c*). The basal levels of $^{32}P_i$ incorporation into rhodopsin in picomoles were 0.33 ± 0.02, 0.33 ± 0.03, and 0.50 ± 0.06 for βARK1, construct 2, and construct 4, respectively. These results suggests that this 28-amino acid truncated βARK1

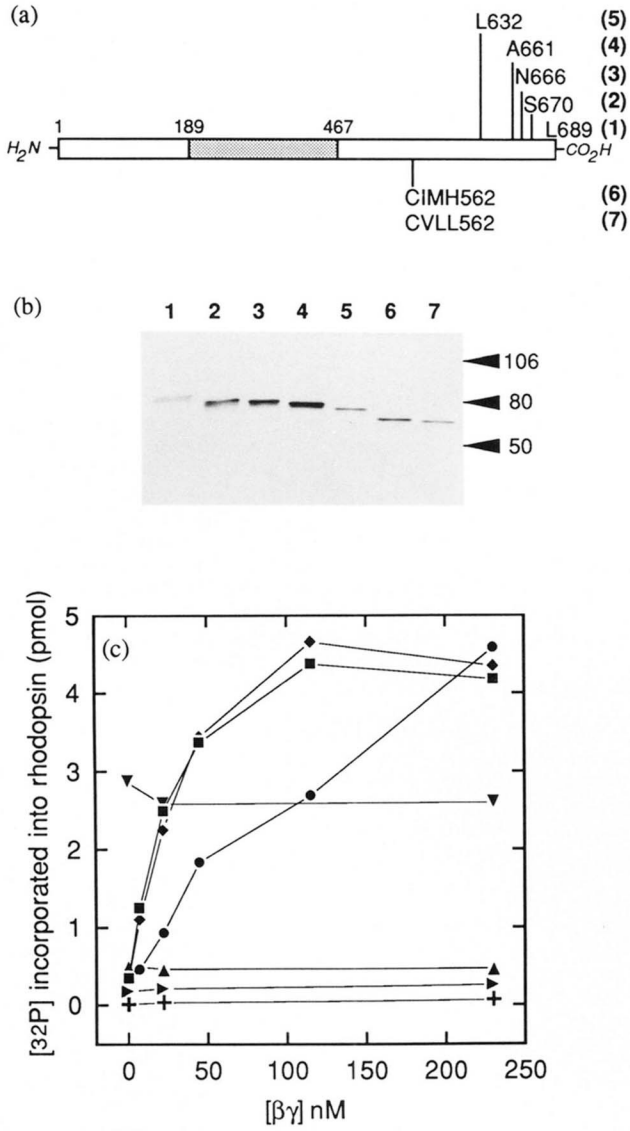

FIG. 1. **Analysis of βARK1 carboxyl-terminal truncations.** *a*, schematic representation of wild-type βARK1 (*construct 1*) and six carboxyl-terminal truncation mutants (*constructs 2–7*). The terminal amino acid residue of each individual mutant is listed in standard single letter code along with it's corresponding βARK1 residue number. The last 4 amino acids of constructs 6 and 7 are listed to show the naturally occurring end of construct 6 and the substituted isoprenylation signal (*CVLL*) on construct 7. *b*, Western blot analysis of the above-described βARK1 truncation mutants (*lanes 1–7*) as expressed in Cos 7 cells (see "Experimental Procedures"). Molecular weight standards (in kilodaltons) are shown to the *right*. *c*, βγ activation profiles for the COS 7-expressed wild-type βARK1 and mutant enzymes (■, construct 1; ◆, construct 2, ●, construct 3; ▲, construct 4; +, construct 5; ▶, construct 6; ▼, construct 7). Increasing concentrations of brain βγ were added to a phosphorylation mixture containing the kinase and ROS membranes (see "Experimental Procedures"). Data shown are the mean specific $^{32}P_i$ incorporation values (after subtraction of the nonspecific phosphorylation produced by COS 7 supernatants transfected with empty vector) of at least three separate experiments.

mutant kinase (construct 4) still has normal catalytic activity and is only lacking in its βγ-activation properties. The basal and βγ-stimulated activities of the βARK1 19- and 28-amino acid truncation mutants indicate that the distal boundary of the domain responsible for the βγ interaction is in the area spanning the 9 amino acid residues between the termini of these two mutants (Ala^{661} to Ser^{670}). A 23-amino acid truncation was constructed and expressed (Fig. 1*a*, *construct 3*) to further define the boundary, and as shown in Fig. 1*c*, this mutant displays an intermediate βγ activation profile. A similar maximal βγ response is seen with this mutant, but the apparent affinity for βγ is lower than that of the wild-type kinase and 19-amino acid truncation mutant. The concentration of βγ required to effect half-maximal activation of these three kinases (present at a concentration of 20 nM) were as follows: wild-type βARK1, 25 ± 8 nM, the 19-amino acid truncation mutant, 41 ± 10 nM, and the 23-amino acid truncation mutant, 126 ± 43 nM. These results indicate that the region responsible for activation by βγ has been disrupted in the 23-amino acid truncation mutant, and we conclude that the distal end of this domain is approximately at βARK1 residue Ser^{670}.

Interestingly, truncating a total of 57 amino acids from the carboxyl terminus of βARK1 (Fig. 1*a*, *construct 5*) results in a mutant enzyme which not only fails to undergo βγ activation but also has significantly diminished basal activity (Fig. 1*c*), suggesting that sequences within the last 57 amino acids may be required for proper protein folding or perhaps the interaction of the kinase with the activated membrane bound receptor substrate. A further truncation to 127 amino acids (Fig. 1*a*, *construct 6*) produced essentially the same results as the 57-residue truncation (Fig. 1*c*). Surprisingly, the substitution of the isoprenylation signal CVLL (directing geranylgeranylation) (Fig. 1*a*, *construct 7*) in place of the last 4 residues of construct 6 (CIMH), restored activity of this markedly truncated kinase to within a factor of two of the activity which is observed when wild-type βARK1 is stimulated by βγ (Fig. 1*c*). βγ addition to this geranylgeranylated truncated kinase did not further increase the activity. These data support the hypothesis that, as suggested previously (16), isoprenylation of βARK1 can partially fulfill the function served by the βγ interaction by providing a suitable membrane anchor.

βγ Binding to GST-βARK Fusion Proteins—With the distal end of the βγ activation domain localized, a second approach, measuring the direct binding of βγ to βARK, was used to determine the complete region. Direct βγ binding can be observed using GST-βARK fusion proteins, and we have reported previously (15) that βγ specifically binds to βARK1 within the large carboxyl third of the enzyme. Using the GST-βARK1 fusion proteins displayed in Fig. 2*a*, we have identified the minimal region within the carboxyl terminus of βARK required for high-affinity βγ binding. The specific binding of βγ to the various GST-βARK fusion proteins was examined first by an incubation with purified βγ subunits followed by the addition of glutathione-Sepharose beads. After extensive washing of the immobilized GST-βARK fusion proteins, retention of βγ subunits was assessed by probing a Western blot of the βARK fusion protein-βγ complexes with β antibodies (Fig. 2*b*).

Using this assay, we were able to systematically remove amino-terminal portions of the carboxyl-terminal domain of βARK1. βγ binding was observed in a GST-βARK1 fusion protein which started at Val^{525} eliminating the first 58 amino acids of the carboxyl domain of βARK1 (Fig. 2*b*, *II*). βγ binding was observed with this fusion protein possessing the wild-type carboxyl terminus. Also, consistent with our above findings in COS cells, a fusion protein in which the final 19 amino acids were truncated from the carboxyl terminus still bound βγ (Fig. 2*b*, *V*), whereas the corresponding fusion protein with the terminal 28 residues truncated displayed significantly lower amounts of βγ binding (Fig. 2*b*, *VI*). Continued paring at the amino end of the fusion proteins indicated that the minimal length necessary to bind βγ as completely

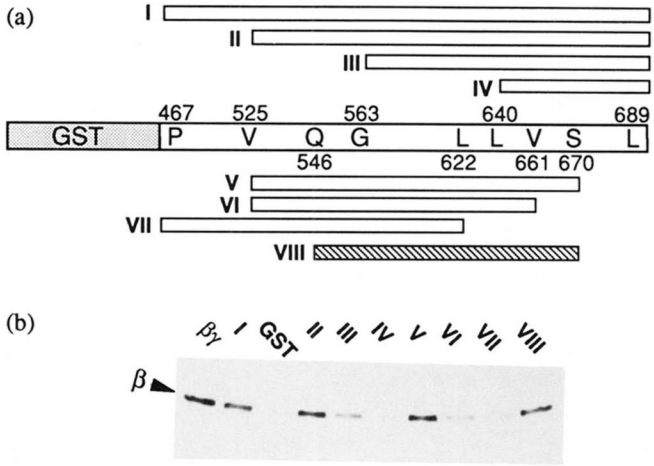

FIG. 2. **Analysis of GST-βARK1 fusion proteins.** *a*, diagrammatic representation of the eight βARK1 carboxyl-terminal segments which were fused to glutathione *S*-transferase and expressed in *E. coli* as described under "Experimental Procedures." Each GST-βARK1 fusion protein (*I–VIII*) differs in the beginning and ending amino acid residue which is depicted in the *center map* using standard single amino acid code and it's corresponding βARK1 residue number. The *shaded* GST-βARK1 fusion protein (*VIII*) represents the minimal βγ binding domain as determined by direct βγ binding and visualization via Western blotting using β antibodies. The βγ binding properties of all GST-βARK1 fusion proteins are shown in b, where the position of the β subunit is marked on the *left* by an *arrow*. Brain βγ was utilized as a positive control and GST alone as a negative control (see "Experimental Procedures" for details).

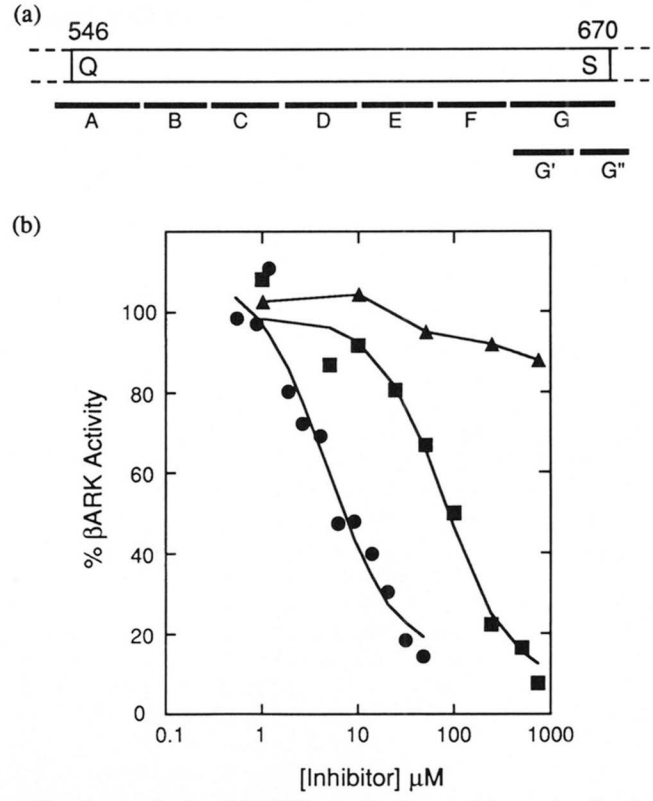

FIG. 3. **Analysis of βARK1 synthetic peptides.** *a*, localization of the βARK1 synthetic peptides *A–G″* as they lie within the above-determined 125-amino acid minimal βγ binding domain. The composition of the peptides encompasses the following amino acid residues: *A*, A531-Y553; *B*, A554-N570; *C*, P571-E589; *D*, W590-S608; *E*, V609-G626; *F*, G627-Q642; *G*, W643-S670; *G′*, W643-L657; and *G″*, V658-V672. *b*, inhibitory dose-response curves for the active βARK1 peptide G (■), the inactive peptide G′ (▲), and the GST-βARK1 fusion protein I (●) against βγ-activated βARK1 phosphorylation activity (see "Experimental Procedures" for assay details). The IC_{50} values (mean ± S.E. of at least three separate experiments) for peptide G and GST-βARK1 fusion protein I are 76 ± 7 and 7.7 ± 3 μM, respectively.

as the full-length carboxyl terminus (Fig. 2*b*, *I*) was a 125-amino acid domain comprised of βARK1 residues Gln^{546} to Ser^{670} (Fig. 2*b*, *VIII*). GST-βARK2 fusion proteins consisting of the complete carboxyl terminus and the smaller 125 amino acid region (βARK2 residues Gln^{546} to Ser^{670}) were also constructed and these fusion proteins displayed the same βγ binding profiles as their βARK1 counterparts (data not shown). A GST-βARK1 fusion protein deleting an additional 17 amino acids from Gln^{546} (to amino acid residue Gly^{563}) displayed a significantly lower amount of βγ retention (Fig. 2*b*, *III*). Fusion proteins consisting of the first 150 amino acids (Pro^{467} to Leu^{622}) and the last 50 amino acids (Leu^{640} to Leu^{689}) of the carboxyl terminus of βARK1 also displayed minimal βγ retention in our assay (Fig. 2*b*, *VII* and *IV*, respectively). It could not be determined using this assay whether these final three fusion proteins did not effectively bind βγ because the domain was actually disrupted (*VII*) or because of possible steric interference with the accessibility of the βγ domain by the larger GST portion of the fusion protein (*III* and *IV*). In any case, this newly identified 125-amino acid βγ binding domain (βARK1 and -2 residues Gln^{546} to Ser^{670}) served as a basis for construction of synthetic peptides to further localize specific βγ binding site(s).

Inhibition of βγ Activation of βARK1 by Synthetic Peptides—To identify short critical regions involved in the βγ binding site, we synthesized a series of peptides (15–28-mers) encompassing the 125-amino acid βγ binding domain of βARK1 and then tested them as inhibitors of βγ activation of βARK1. A map defining these specific peptides is shown in Fig. 3*a*. Peptides A–F (Fig. 3*a*) produced no apparent inhibitory activity of the βγ activation of βARK1 as assessed in ROS membrane phosphorylation assays. The only peptide with specific βγ inhibitory activity was peptide G, a 28-mer peptide corresponding to the βARK1 residues Trp^{643} to Ser^{670} (Fig. 3*a*). This peptide, comprised of the last 28 amino acids of the above described 125 amino acid βγ binding domain, inhibited the βγ activation of βARK1 with a calculated mean IC_{50} of 76 μM. The fact that no other peptide exhibited significant inhibitory activity strongly suggests that the βγ binding domain lies partially or completely within this 28-amino acid stretch.

A dose-response curve of peptide G for the inhibition of the βγ stimulated βARK1 activity is shown in Fig. 3*b* along with dose-response curves of the βARK1 carboxyl-terminal GST-fusion protein (Fig. 2*a*, *I*) and peptide G′, a representative nonactive βARK1 peptide, which is actually the first 15 amino acid residues of peptide G. The inhibitory potency of the GST-βARK1 carboxyl-terminal fusion protein is 1 order of magnitude greater than peptide G with a calculated mean IC_{50} of 7.7 μM. A GST-fusion protein consisting of the βARK2 carboxyl terminus and a βARK2 peptide G were also tested and had similar inhibitory activity against the βγ activation of βARK1 as their βARK1 counterparts (data not shown). As seen in Fig. 3*b*, the inhibition elicited by the fusion protein and peptide G does not reach 100%. This is due to the βγ-independent basal βARK activity described above in Fig. 1*c*.

The sequence of peptide G (WKKELRDAYREAQQL VQRVPKMKNKPRS) does not appear to contain any noteworthy characteristics aside from being basic in nature (pI = 10.75). As mentioned above, shortening peptide G to the first 15 residues (Fig. 3*a*, *G′*) abolished activity. Similarly, peptide G″ (Fig. 3*a*) containing the last 13 amino acids of peptide G

and an additional 2 amino acids was also inactive. The difference in apparent βγ affinity between the entire βARK1 carboxyl terminus and peptide G suggests that the 28 amino acid residues may need to be present in the environment of the complete carboxyl-terminal domain to stabilize the proper conformation for high-affinity βγ binding. Although no other peptides besides peptide G displayed any specific βγ inhibitory activity, the possibility still exists that other residues outside this 28-amino acid region may play a significant role in βγ binding.

An additional interesting phenomenon was revealed during the course of this study which provides possible insight into the evolution of the gene family of G protein-coupled receptor kinases. The other currently known member of this family of kinases besides βARK is RK. The main structural difference between RK and βARK is the length of the carboxyl terminus, with βARK being approximately 125 amino acids longer than RK (21). The 127-amino acid truncated βARK1 mutant described in Fig. 1, *construct 6*, which is inactive, represents an enzyme truncated to the size of RK. The markedly improved activity seen when this mutant was modified by a geranylgeranyl isoprenoid (Fig. 1*a*, *construct 7*) demonstrates that a βARK, not only shortened to mimic RK's length but also isoprenylated, can effectively phosphorylate its receptor substrate. This is due to the membrane-anchoring properties of the artificially acquired integral isoprenyl moiety. Wild-type βARK1 accomplishes membrane localization by binding to βγ, whereas the shorter and homologous RK uses it's naturally occurring integral isoprenoid to translocate to the membrane (15, 16). We speculate that βARK may have evolved from RK by losing its own isoprenylation signal through the acquisition of exons which encode the βγ binding domain enabling the more widely expressed βARK to be tightly controlled in hormone-sensitive signaling systems.

We have utilized three complementary approaches to identify a 28-amino acid domain within the carboxyl terminus of βARK1 and βARK2 which specifically interacts with and binds to the βγ subunits of signal transducing heterotrimeric G proteins. This specific protein-protein interaction appears to take place within the last 28 amino acids of an experimentally determined 125-amino acid βARK βγ binding domain, resulting in the plasma membrane targeting of these cytoplasmic kinases via the membrane anchoring properties of the geranylgeranylated γ subunit. This novel action of βγ results in the increased phosphorylation of the β-adrenergic and other related G protein-coupled receptors initiating the desensitization of these hormone receptors.

It will be interesting to learn if specific βγ complexes will have distinct affinities for βARK1 or βARK2. Specificity within this βARK-βγ interaction would allow for highly tuned regulation in cell types expressing different levels of specific receptor subtypes which may be coupled to unique G proteins resulting in the liberation of specific "βγ pools." It has been shown recently that different β subunits can direct coupling to distinct G protein-coupled receptors (6). Thus it appears that different βγ pools could preferentially translocate/activate specific kinases to turn off their respective signals.

The βγ binding domain on βARK1 and -2 which we have defined in this study represents the first such "βγ effector" domain to be delineated. βARK, however, is not a classical effector enzyme as its action does not amplify the hormone signal, but rather the increased receptor phosphorylation directed by the βARK-βγ interaction turns off the signal. Other effector molecules which appear to be directly modulated by βγ, such as type II adenylate cyclase (8, 9), are enzymes which are responsible for producing the cellular responses seen as a result of hormone action. The mechanism of action of βγ on other G protein-coupled enzymes is not yet clear, but the definition of the βARK βγ binding domain and more importantly the availability of peptides such as the βARK1 peptide G should prove valuable in searching for the βγ interaction domains within other G protein-coupled effector molecules. It seems probable that peptides such as those described here might function as specific or more general inhibitors of the various effector functions of βγ. In the case of the βARKs, they provide a novel approach to inhibiting the receptor desensitization mediated by the enzymes and hence a potential starting point for the development of therapeutic agents directed at this purpose.

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