A Domain on the G Protein β Subunit Interacts with Both Adenylyl Cyclase 2 and the Muscarinic Atrial Potassium Channel*  

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The G protein βγ complex modulates the function of a variety of effectors in biological signaling. However, the individual roles of the β and γ subunits in this interaction are unknown. Unlike in the case of the α subunit, domains on the βγ complex that contact effectors have not yet been identified. We show here using the yeast two-hybrid system that the β subunit and not the γ subunit interacts with domains specific to adenylyl cyclase type 2 (AC2) and the muscarinic receptor-gated atrial inwardly rectifying potassium channel, GIRK1. Different β subunit types interact with these effector domains with different efficiencies. Furthermore, an N-terminal fragment of 100 residues interacts with both these effector domains as effectively as the whole β subunit. This domain includes the region where the β subunit contacts with the α subunit in the crystal structure and may therefore explain the ability of the α subunit to shut off the activity of the βγ complex.

Both the α subunit and the βγ complex of a heterotrimeric G protein are capable of regulating the function of a variety of effectors (1). Among the effectors regulated by the βγ complex are adenylyl cyclases, phospholipase C-β, and potassium channels (2–6). The βγ complex also interacts with the β-adrenergic receptor kinase (7, 8). Several studies have now shown that the βγ complex acts on these molecules directly (9–16). Since the evidence so far indicates that the β and γ subunits are tightly bound as a complex, it has not been clear whether one or both subunits interact with effectors. Several other questions have also remained. What are the relative positions of sites on βγ that interact with unrelated effectors such as adenylyl cyclases and potassium channels? What is the mechanism that allows βγ to be switched off after acting on an effector? Is there specificity of interaction between βγ subtypes and effectors since both the β and γ subunits are families of proteins which are capable of combining to form a variety of complexes (17, 18)?

We have recently shown that G protein β and γ subunit types effectively interact with each other as fusion proteins in the yeast two-hybrid system (19). These studies also showed that the reporter activity elicited by particular combinations of β and γ subunit types was directly related to the amount of that complex formed in the cell as determined by immunoprecipitation. We therefore used the two-hybrid system to examine interaction of the βγ complex with effectors. We chose to analyze interactions of βγ with adenylyl cyclase type 2 (AC2) and the muscarinic receptor-gated atrial inwardly rectifying potassium channel (GIRK1) since domains on these effectors which interact with βγ had been identified (11–14). A 27-residue peptide specific to the second of the two large cytoplasmic loops of AC2 was shown to be capable of inhibiting the ability of the βγ complex to regulate several effectors, AC2, AC1, K+ channels, phospholipase C-β, and the β-adrenergic receptor kinase (20). These results indicated that a single site on the βγ complex could potentially interact with various effectors. Two sites on GIRK1 that interact with βγ have been identified (11–14). One of these sites lies at the N terminus of GIRK1 and includes the first 83–85 residues. Fusion proteins including this domain bind to the βγ complex and a peptide from this region inhibits βγ complex activation of the atrial K+ channel (13, 14). We have examined the interaction of these effector domains from adenylyl cyclase 2 and GIRK1 with all known β subunit types, β1–β5, and five of the γ subunits, γ2, γ3, γ4, γ5, and γ7 (19).

MATERIALS AND METHODS

Strains—Yeast strain Y190 and the plasmids pAS I and pACT II have been described (19). pSA1 contains the Gal4 binding domain and pACT II, the activation domain. cDNAs for γ2, γ3, γ4, γ5, and γ7 subunits were subcloned into pAS I and cDNAs for β1–β5 subunits into pACT II, as described (19). A synthetic oligonucleotide cassette encoding the AC2Q domain (amino acids 956–982 of adenylyl cyclase 2) was subcloned into both pAS I and pACT II using Ncol/BamHI. A similar cassette encoding AC2Q* (a scrambled sequence of AC2Q) was subcloned into pASI using Ncol/BamHI. DNA encoding the GKN domain (residues 1–83 of GIRK1) was amplified by polymerase chain reaction and subcloned into both pAS I and pACT II using Ncol/BamHI. cDNA fragments for four regions of β1 were subcloned as follows. β1A (residues 1–100), an EcoRI/BspHI fragment filled with Klenow, was inserted into the Ncol site of pACT II filled with Klenow, β1B (residues 101–187) and β1C (residues 188–261), two BspHI fragments, were inserted into the Ncol site of pACT II. β1D (residues 188–340), a BspHI/EcoRI fragment, was inserted into Ncol/EcoRI-cut pACT II. Correct reading frames of all of the constructs mentioned above were confirmed by nucleotide sequencing. Control plasmids expressing BD-Tat and AD-G1 have been described (19).

Assay of β-Galactosidase Activity and Immunoblot Analyses—For the filter assay of β-galactosidase activity, yeast transformants were streaked on a white filter paper that was laid on a synthetic medium plate lacking Trp and Leu and incubated at 30 °C overnight. The filter was treated with liquid nitrogen for 10 s and laid on a Whatman No. 5 filter presoaked with Z buffer containing 5-bromo-4-chloro-3-indolyl β-D-galactoside for 2 h (Matchmaker Library Protocol, Clontech, Palo Alto, CA). The liquid culture assay of β-galactosidase activity and immunoblotting have been described previously (19). Expression of the fusion proteins was detected with Gal4-specific antibodies (1 μg/ml) (Clontech, Palo Alto, CA).

RESULTS AND DISCUSSION

We first tested the interaction between the β1 subunit, a β subunit type that is expressed at high levels in all mammalian tissues, and the AC2 domain AC2Q identified by Chen et al. (20). β1 was a hybrid with the activation domain (AD)2 of the

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Gal4 transcription factor, and the AC2Q domain was a hybrid with the binding domain (BD). Plasmids expressing these hybrids were co-transformed into a yeast strain containing the gene for the reporter, β-galactosidase (β-gal), under the control of the GAL promoter. β-gal activity was measured spectrophotometrically and compared with various other combinations of hybrids (Fig. 1A). β1+ACQ elicited almost as much activity as β1+γ5, a γ subunit type that interacts strongly with β1 (19). ACQ did not interact with γ5 or an unrelated control, Grr1. β1 did not interact with an unrelated control protein, Tat, as we have shown previously (19). To examine whether the interaction between the β subunit and the adenylyl cyclase domain is specific, the β1 subunit was co-expressed with ACQ* which has the amino acid sequence of ACQ scrambled. This combination did not produce any reporter activity (Fig. 1B). We then examined the interaction of the AC2Q domain with all the β subunit types identified so far. The reporter activity produced by various combinations was different, although the maximum difference was about 4-fold (between β1 and β4) (Fig. 1B). None of the γ subunit types tested interacted with the AC2Q domain (Fig. 1C), consistent with the inability of γ5 to do so. None of the β subunit types interacted significantly with the AC2Q* domain (data not shown). The differences in the reporter activity elicited by various β subunit types in combination with AC2Q were not due to differences in the expression levels of β subunit hybrids or the AC2Q hybrid (Fig. 2, A–C). Although the differential activity could result from inadequate folding or access to the nucleus, it is more likely due to differential interaction between β subunit types and AC2Q since reporter activity elicited by hybrids has been shown to be directly related to the efficacy of complex formation between those hybrids (19).

In a study examining the ability of different βγ subtypes to activate AC2 significant differences among subtypes were not seen (21). The combinations tested in this study were made up of β1 or β2 which did not show significant differences in their interaction with the AC2Q (Fig. 1B). Based on our results (Fig. 1B), it is possible that βγ complexes containing β4 may be less effective in activating AC2 than those containing β1 or β2.

Since the G protein β subunits interacted with the AC2 domain, we tested their ability to interact with the N-terminal domain of GIRK1 (GKN). This domain can interact with the βγ complex (13, 14). Fig. 3 shows the β-gal activity in liquid cultures (Fig. 3A) and on filters (Fig. 3B), from transformants co-expressing different β subunit types and GKN. The β1 and β2 subunits interacted with GKN while the others did not. None of the γ subunits interacted with GKN (Fig. 3C). The reporter activity elicited by β1 or β2 with GKN was distinctly less than that produced with AC2Q. However, the extent of reporter activity was significant compared with a variety of controls, indicating effective interaction between GKN and the two β subunit types. It is of interest that only the β1 and β2 subunit types showed significant interaction with GKN. Experiments examining the activity of βγ subtypes on the muscarinic K+ channel did not detect significant differences among the various complexes (22). However, all the βγ complexes examined in these experiments contained either β1 or β2, which did not differ in interaction with GKN in our assays (Fig. 3A and B). Based on our results (Fig. 3, A and B), it is possible that βγ complexes containing β3, β4, or β5 subunit types would show significant differences in activating the K+ channel.
Materials and Methods.

These results indicated that the activity determined from three different transformants examined in each of two independent experiments. Filter assay is representative of β1A-D, with the AC2Q domain (Fig. 4C). The β1A fragment interacted as efficiently with AC2Q as the whole β subunit. β1B-D showed little or no interaction with AC2Q. None of the fragments interacted with the AC2Q domain (data not shown). Although this strongly supports the notion that the N-terminal 100 residues of the β subunit contains a site that interacts with AC2Q, it does not rule out the possibility that the other fragments also have sites that interact with AC2Q which are concealed because these fragments are folded improperly. However, considering that the AC2Q domain is relatively small, it is unlikely that it interacts with sites that are widely separated in the crystal structure of the β subunit. Since a peptide specific to the AC2Q domain has been shown to be capable of inhibiting the activation of atrial K⁺ channels by the βγ complex (20), we tested whether β1A also interacts with the GKN domain. The results are shown in Fig. 5, A and B. The β1A fragment interacts with GKN with almost the same efficiency as the whole β subunit while the other fragments, β1B-D, did not show any significant interaction. As in the case of AC2Q, these results do not rule out the possibility that the β1B-D fragments also have interaction sites for the GKN domain since we do not have direct evidence that those fragments are folded appropriately.

Based on the secondary structure of the β subunit deduced by crystallography, the N-terminal 100 residues of the β subunit can be divided into a helix (that forms a coiled coil with the N-terminal helix of γ), a loop, and five β strands (23, 24). The first of these β strands is the outer strand of the “seventh” β sheet, in the torus-like tertiary structure of the β subunit which is made up of seven β sheets. The next four strands form the “first” sheet. The region containing these five β strands is thus in close proximity to the N-terminal portion of the α subunit. It has been shown in several systems that effector activation by the βγ complex is switched off by heterotrimerization with the α subunit (e.g. Refs. 2, 4, 5, and 22). The ability of β1A to interact with AC2Q and GKN is thus consistent with the ability of the α subunit to displace the effectors. Consistent with this, cross-linking of a peptide specific to the AC2Q domain to the β subunit is prevented by the α subunit.2 In contrast, the GKN domain has been shown to be capable of

2 R. Iyengar, personal communication.
interaction of various regions of the G protein β1 subunit with the GKN domain. A and B, details are as in the legend to Fig. 3.

Two different domains at the C terminus of GIRK1 have been shown to interact with the G protein βγ complex (12-14). A C-terminal domain of β-adrenergic receptor kinase has also been shown to be capable of interaction with the βγ complex (16). These effector domains did not interact with either the β1 subunit or β1A (data not shown). Although the possibility that these effector domains are misfolded cannot be ruled out, based on these results, we favor a model in which the G protein βγ complex contains at least two sites that interact with G protein effectors, a first site on the β1 domain and a second that remains unidentified. Since the prenyl group is required for effector interaction (5, 11, 21, 26), one possibility is that this second site includes the prenyl moiety at the C terminus of γ subunits. The prenyl group is expected to be positioned close to the α subunit N terminus and the C-terminal half of β1A in the crystal structure (23, 24). This would explain the ability of the α subunit to disrupt βγ interaction with effectors and also explain the ability of a peptide specific to the AC2Q domain to inhibit βγ regulation of a variety of effectors (20).

The identification of a site for effector interaction on the G protein β subunit is the first indication that it plays a direct role in the modulation of effector function. Previously, a direct role for the γ subunit in receptor interaction has been shown (27, 28). Together these results indicate that the G protein βγ complex possesses two important functions localized in the subunits that make up the complex, the γ subunit directing specific receptor interaction of the heterotrimer and the β subunit specifying effector interaction of the βγ complex. There are indications, however, that these functions may be overlapping since prenylation of the γ subunit is a requirement for effector interaction of the βγ complex (5, 11, 21, 26), and cross-linking studies indicate that the β subunit is in contact with the receptor (29).

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