

Receptor and Membrane Interaction Sites on $G\beta$

A RECEPTOR-DERIVED PEPTIDE BINDS TO THE CARBOXYL TERMINUS*

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Joan M. Taylor‡, Gayatry G. Jacob-Mosier§, Richard G. Lawton§, Marcian VanDort¶, and Richard R. Neubig‡||**

From the Departments of ‡Pharmacology, §Chemistry, ¶Nuclear Medicine, and ||Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109

The functional organization of $G\beta\gamma$ is poorly understood. Regions of bovine brain $G\beta\gamma$ that interact with a photoaffinity derivative of an α_2 -adrenergic receptor-derived peptide from the third intracellular loop (diazopyruvoyl-modified peptide Q (DAP-Q)) and a hydrophobic membrane probe (3-trifluoromethyl-3-(*m*-iodophenyl)diazirine (TID)) were examined. We previously showed that DAP-Q cross-links to specific, competent sites on both the α and β subunits of G_o/G_i but not on the γ subunit and that $\beta\gamma$ subunit was required for stimulation of G_o/G_i GTPase activity (Taylor, J. M., Jacob Mosier, G. G., Lawton, R. G., Remmers, A. E., and Neubig, R. R. (1994) *J. Biol. Chem.* 269, 27618–27624). Similarly, we show here that the membrane-associated photoprobe [125 I]TID labels α and β but not γ . We have now mapped the sites of incorporation of DAP-Q and TID into the β subunit. TID labels both the 14-kDa amino-terminal and the 23-kDa carboxyl-terminal fragments from a partial tryptic digest of β while DAP-Q labels only the carboxyl-terminal fragment. Further mapping with endopeptidase Lys C reveals substantial labeling of multiple fragments by TID while DAP-Q labels predominantly a ~6-kDa fragment within the carboxyl-terminal 60 amino acids of β . Thus, regions within the 7th (or possibly 6th) WD-40 repeat of the β subunit of G protein interact with the receptor-derived peptide while membrane interaction involves multiple sites throughout the β subunit.

Heterotrimeric G proteins (composed of α , β , and γ subunits) transmit intracellular signals from a family of plasma membrane-associated G protein-coupled receptors (GPCR).¹ This family includes adrenergic receptors, photoreceptors, and

growth factor receptors among others. Binding of ligand causes a conformational change in the receptor, which activates the associated G protein. The activated G protein dissociates into an α subunit and a $\beta\gamma$ subunit complex. Both the α and $\beta\gamma$ subunits are able to activate intracellular effector enzymes (1).

The GPCRs have seven transmembrane helices with three cytoplasmic loops and an intracellular carboxyl terminus (2). Mutagenesis (3, 4) and competition studies using synthetic peptides (5–9) suggest that the i3 loop and possibly the second cytoplasmic loop and carboxyl-terminal tail are important for receptor-G protein interactions. Peptide Q is a tetradecapeptide from the carboxyl-terminal part of the i3 loop of the α_2 AR. Peptide Q can inhibit α_2 AR- G_i coupling and can also mimic GPCRs by binding to and activating G protein directly (5, 8, 10).

We have previously described a photoaffinity label (DAP-Q) prepared by coupling the sulfhydryl-reactive Br-DAP to the G protein activator peptide (peptide Q) (11). DAP-Q and a radioiodinated derivative ([125 I]pHBDAP-Q) cross-link at nanomolar concentrations to specific, competent sites on both the α and β subunits but not on the γ subunit of G_o/G_i (12). Also, a functional interaction between DAP-Q and $\beta\gamma$ is required for DAP-Q-stimulated GTPase activity. Binding of DAP-Q to the amino terminus of α (12) is consistent with a number of reports implicating the closely associated amino and carboxyl termini of α subunits in binding receptor (9, 13–17).

In addition to its role in membrane association of α subunits (18), much recent evidence supports a direct interaction of the $\beta\gamma$ subunit complex in the coupling of receptors to G proteins. Binding of purified β_1 -adrenergic receptors and rhodopsin with their respective G protein $\beta\gamma$ subunits has been demonstrated (19, 20). Kleuss *et al.* (21, 22) have shown that antisense probes directed against the β_3 subtype or the γ_4 subtype can block muscarinic receptor inhibition of calcium currents while probes directed against β_1 or γ_3 block somatostatin receptor inhibition of calcium currents (21, 22). Also, Kisselev and Gautam (23) have shown that rhodopsin binds to a G protein containing γ_1 but not γ_2 or γ_3 subunits. The isoprenoid-modified carboxyl terminus of γ subunit is important for coupling to rhodopsin (24).

The β subunit is composed of seven highly conserved WD-40 repeat regions characterized by a Gly-His followed by 23–41 core amino acids and a WD (Trp-Asp) (25). The function of these WD-40 repeats is not known, but it has been proposed that the repeats are important for protein-protein interactions (25). The specific regions of β subunits participating in either receptor coupling or membrane association remain to be identified. In this report we have mapped the major binding site on the β subunit for the G protein activator peptide (DAP-Q) and have begun to localize labeling sites for the membrane-associated photoprobe [125 I]TID.

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** To whom correspondence should be addressed: Dept. of Pharmacology, University of Michigan, 1301 MSRBIII, Ann Arbor, MI 48109-0632. Tel.: 313-763-3650; Fax: 313-763-4450; E-mail: RNeubig@umich.edu.

¹ The abbreviations used are: GPCR, G protein-coupled receptor; AR, adrenergic receptor; Br-DAP, *N*-bromoacetyl-*N'*-(3-diazopyruvoyl)-*m*-

phenylenediamine; DAP, diazopyruvoyl; DAP-Q, DAP-modified peptide Q; G_o , abundant G protein purified from bovine brain; G_i , inhibitory G protein purified from bovine brain; pHBDAP, *N*-bromoacetyl-*N'*-(4-hydroxybenzyl)-*N'*-(3-diazopyruvoyl)-1,3-phenylenediamine; pHBDAP-Q, pHBDAP-modified peptide Q; i3, third intracellular loop; peptide Q, peptide with the sequence RWRGRQNREKRFTC (amino acids 361–373) from the porcine α_2 AR-adrenergic receptor with additional carboxyl-terminal cysteine; TID, 3-trifluoromethyl-3-(*m*-iodophenyl)diazirine; HPLC, high pressure liquid chromatography; PAGE, polyacrylamide gel electrophoresis; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; GTP γ S, guanosine 5'-3'-O-(thio)triphosphate.

MATERIALS AND METHODS

Chemicals—Br-DAP and the iodinated *p*-hydroxybenzyl derivative of Br-DAP, *N*-bromoacetyl-*N*-(4-hydroxybenzyl)-*N'*-(3-diazopyruvyl)-1,3-phenylenediamine (pHBDAP), were synthesized as described (11, 12, 26). pHBDAP was radioiodinated with Na¹²⁵I by the chloramine-T method as described previously (27, 28). To permit a significant stoichiometry of labeling of $\beta\gamma$ by [¹²⁵I]pHBDAP-Q to reduce complications of unmodified β subunits, we isotopically diluted the cross-linker with unlabeled pHBDAP-Q 30-fold from its original specific activity of 2200 to ~73 Ci/mmol. [¹²⁵I]TID (10 Ci/mmol) was from Amersham Corp., and the carboxyl-terminal β subunit antibody (SW/1) was from DuPont NEN. Non-radioactive TID was a gift of Dr. Jonathan B. Cohen (Harvard Medical School). HPLC solvents were from J. T. Baker Inc. All other chemicals and reagents were reagent grade or better and were purchased from Sigma.

Peptide Synthesis—Peptide Q (RWRGRQNREKRFTC) was synthesized by a Biosearch 9600 peptide synthesizer using Fmoc (fluorenylmethoxycarbonyl) chemistry and purified by a preparatory HPLC Beckman System Gold on a reverse phase column. Peptide Q corresponds to the carboxyl-terminal region of the i3 loop of the porcine α_2A AR (residues 361–373). The peptide has an additional cysteine attached to the carboxyl terminus of the native receptor sequence. The purity and identity of the peptide was confirmed by mass spectroscopy using a Vestec single quadrupole mass spectrometer with electrospray interface by the Protein and Carbohydrate Structure Core at the University of Michigan.

G Protein Purification and Preparation—G_o/G_i was purified from synaptosomal membranes of bovine brain cortex by the method of Sternweis and Robishaw (29) as modified by Kim and Neubig (30). Protein was quantitated using the method of Schaffner and Weissmann (31). [³⁵S]GTP γ S binding was measured as described (29). To obtain purified α and $\beta\gamma$ subunits, the G protein was activated with AlCl₃, MgCl₂, and NaF as described elsewhere (32, 33). The subunits were then separated as described by Kwon *et al.* (34) using heptylamine-Sepharose chromatography.

Photolysis of DAP-Q with Purified G Protein Preparations—Peptide Q was conjugated to Br-DAP or [¹²⁵I]pHBDAP, and the complex was purified by HPLC as described previously (11). DAP-Q or [¹²⁵I]pHBDAP-Q was incubated on ice for 10 min with purified $\beta\gamma$ subunit or G_o/G_i from bovine brain at the indicated concentrations in buffer A (50 mM Na-HEPES, pH 8.0, 1 mM EDTA, 1.3 mM MgCl₂, 0.1% Lubrol, and 60 μ M GTP). A 366-nm mineral light (model UVGL-25) was then placed 4.5 cm from the samples for 10 min. SDS-PAGE sample buffer was then added, and cross-linked products were prepared for electrophoresis.

[¹²⁵I]TID Labeling of G Protein in Membranes—G_o or $\beta\gamma$ subunits were reconstituted by gel filtration into azolectin vesicles as described (12). For labeling, 1 μ M [¹²⁵I]TID with or without 100 μ M unlabeled TID was added to reaction tubes from a stock solution in ethanol. The ethanol was dried with a stream of N₂ in dim light, and then the G protein vesicles were added. Samples were vortexed and then incubated for 10 min on ice prior to photolysis for 30 min as described for DAP-Q (above).

Proteolytic Digestion of the Cross-linked Products—Partial tryptic digestion of β subunit to cleave at Arg¹²⁹ was performed as described (35). Briefly, purified $\beta\gamma$ (100 nM or 0.85 μ g/lane) was incubated with TPCK-treated trypsin (1:100, w/w) for 5–30 min at room temperature. The reaction was stopped by the addition of soybean trypsin inhibitor (1:10, w/w). Trypsin-treated $\beta\gamma$ was photolyzed with DAP-Q (1 μ M) or [¹²⁵I]TID as described above. The digestion products were analyzed by 16% Tricine gel electrophoresis.

Proteolytic cleavage with Lys C was performed using the Cleveland method (36). [¹²⁵I]pHBDAP-Q (3 μ M) or 1 μ M [¹²⁵I]TID was photolyzed with purified $\beta\gamma$ (14 μ M or 50 μ g/lane) in 0.1% Lubrol or azolectin vesicles, respectively. The labeled β subunit was separated from the γ subunit and non-incorporated label by 12.5% SDS-PAGE. The β subunit was identified by staining control lanes with Coomassie Blue, and the corresponding regions of the unstained radiolabeled sample were excised. The excised bands were then applied to a 16% Tricine gel in the presence of endoproteinase Lys C (20:1, protein:enzyme), and the gel was electrophoresed according to the method of Cleveland (36).

SDS-Polyacrylamide Gel Electrophoresis and Western Blots—Cross-linked products were separated on 10 or 12.5% sodium dodecyl sulfate-polyacrylamide gels prepared according to Laemmli (37) or 16% Tricine-polyacrylamide gels (38). For detection of radioactivity, wet or dried gels were exposed on a Molecular Dynamics PhosphorImager. For Western blots, proteins were transferred to Immobilon, labeled with anti- β subunit antiserum (SW/1) at 1/1000 dilution, and visualized as described (12). Alternatively, blots were stained overnight with colloidal gold protein stain (Bio-Rad).

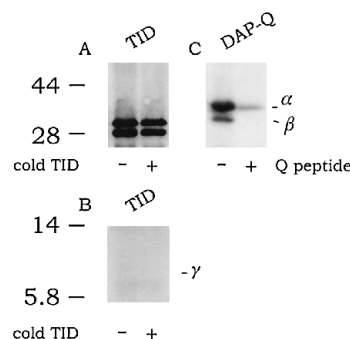


FIG. 1. Incorporation of [¹²⁵I]TID or [¹²⁵I]pHBDAP-Q into G protein subunits. G_o was labeled in azolectin vesicles with 1 μ M [¹²⁵I]TID (A and B) or in Lubrol with 3 μ M [¹²⁵I]pHBDAP-Q (~73 Ci/mmol) (C) as described under "Materials and Methods." [¹²⁵I]TID-labeled samples (A and B) were separated on a 16% Tricine gel, and [¹²⁵I]pHBDAP-Q-labeled samples (C) were separated on a 10% Laemmli gel. Radioactivity was detected with a PhosphorImager. Excess non-radioactive TID (100 μ M) or peptide Q (750 μ M) was added where indicated to test specificity. A and B are from the same exposure of a single gel indicating that labeling of γ subunit is minimal compared with that of α and β .

RESULTS AND DISCUSSION

Several lines of evidence indicate that the $\beta\gamma$ subunit complex binds GPCRs and is important for signal transduction (19–24, 35). We recently reported that the photoactive α_2A AR-derived i3 loop peptide, DAP-Q, labels a highly specific site within the amino-terminal 17 residues of α_o (12). DAP-Q also cross-links to a specific site on the β subunit (12) (see Fig. 1C), and we use this specific labeling to map a potential receptor-interacting region of the β subunit. In contrast to the specificity of labeling by DAP-Q, labeling of α and β subunits by the membrane-associated photoprobe [¹²⁵I]TID is not blocked by excess unlabeled compound (Fig. 1A). This lack of competition is characteristic of membrane-exposed sites (39, 40). Interestingly, the γ subunit did not label with [¹²⁵I]TID (Fig. 1B). While the isoprenoid tail of γ is required for $\beta\gamma$ association with membranes (41), it is possible that the lipid tail is not sufficiently large or reactive to incorporate significant [¹²⁵I]TID, or it may be involved in protein folding or conformation rather than direct lipid interactions.

The functional significance of the specific DAP-Q binding site(s) on $\beta\gamma$ subunit is supported by the absolute requirement of the $\beta\gamma$ subunit for stimulation of α_o subunit GTPase activity (12). Thus, to begin to map the major binding sites on β for DAP-Q and TID we examined their cross-linking to trypsin-treated $\beta\gamma$ subunit. Trypsin treatment of native $\beta\gamma$ subunit results in cleavage of β at Arg¹²⁹, which generates a 14-kDa amino-terminal fragment and a 23-kDa carboxyl-terminal fragment. Thomas *et al.* (35) have shown trypsin treatment of native $\beta\gamma$ subunits does not disrupt tertiary structures or the ability of the complex to associate functionally with the α subunit. For this experiment, the ability of DAP-Q to induce a gel shift of the G protein β subunit was utilized. Fig. 2 shows that DAP-Q photolabels the 23-kDa carboxyl-terminal trypsin fragment (A) but not the 14-kDa fragment of the β subunit (B). It is also evident from Fig. 2A that DAP-Q cross-links equally efficiently to the 23-kDa fragment of trypsin-treated β and to the uncleaved β subunit.² In contrast, [¹²⁵I]TID labels both the 14- and 23-kDa fragments (Fig. 2C). Quantitation reveals $23 \pm 4\%$ ($n = 2$) as much labeling of the 14-kDa fragment as the

² Notably, both 2- and 4-kDa band shifts are observed for the β subunit and the 23-kDa fragment. The double incorporation of DAP-Q (4-kDa band shift) is generally observed when cross-linking is done with probe concentrations of 1 μ M and above. With lower concentrations only single incorporation (a 2-kDa band shift) is observed (12).

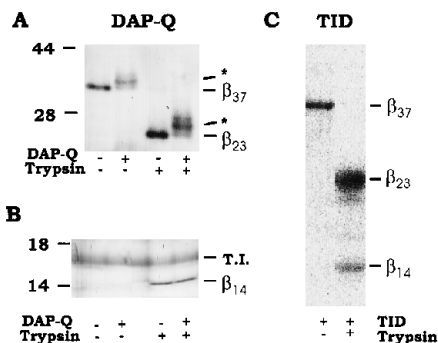


FIG. 2. Photolabeling trypsin-digested $\beta\gamma$ subunit with DAP-Q. Purified $\beta\gamma$ (100 nM or 0.85 $\mu\text{g}/\text{lane}$) in Lubrol (A, B) or azolectin (C) was digested with TPECK-treated trypsin (1:100, w/w) as described under "Materials and Methods." After 30 min, the reaction was stopped by adding soybean trypsin inhibitor (10:1, inhibitor:trypsin). The trypsin digest was then photolyzed with DAP-Q (1 μM) or 1 μM [^{125}I]TID. The products were separated by 16% Tricine gel electrophoresis, transferred to Immobilon, and analyzed by Western blot using a carboxyl-terminal anti- β antibody (A), stained with colloidal gold (B), or scanned with a Molecular Dynamics PhosphorImager (C). The data are representative of four (A and B) or two (C) separate experiments. β_{37} indicates the position of the native (37 kDa) β subunit, whereas β_{23} and β_{14} indicate the positions of the carboxyl-terminal 23-kDa fragment and the amino-terminal 14-kDa fragment. * indicates the position of a DAP-Q cross-linked product. T.I. indicates the position of soybean trypsin inhibitor.

23-kDa fragment, consistent with significant degrees of membrane contact for both fragments.

To further localize the major binding sites on the β subunit we labeled purified $\beta\gamma$ with [^{125}I]pHBDAP-Q in Lubrol and digested the products with endoproteinase Lys C (20:1, protein:enzyme) according to Cleveland *et al.* (36). Fig. 3 shows the results of a partial endoproteinase Lys C digestion of [^{125}I]pHBDAP-Q-labeled β subunit. The majority of the radioactivity migrates as a ~6-kDa fragment of the β subunit (lane A, solid arrow). The silver stain of this gel (lane C) reveals a number of β fragments ranging from 28 to 1 kDa. Complete digestion of the β subunit should result in fragments ranging from 10.4 to 0.4 kDa. Thus the high molecular weight fragments in lane C show that the β subunit does not digest to completion under these conditions. Labeling of β by [^{125}I]TID showed a much more extensive distribution of label (Fig. 3, lane B). The ~6-kDa fragment incorporated a significant amount of [^{125}I]TID, but there were 5 additional fragments clearly labeled.

To identify the position of the ~6-kDa fragment within the sequence of the β subunit, we examined the size of predicted Lys C fragments that overlap the 23-kDa COOH-terminal tryptic fragment of the β subunit. Only labeling within residues 281–341 in β_1 or 302–341 in β_2 predicts labeled fragments smaller than 8.9 kDa (Fig. 4).³ Since labeling within 281–301 (light gray in Fig. 4) would generate a radiolabeled fragment of

³ Matrix-assisted laser desorption mass spectral analysis of a Lys C digestion of our purified $\beta\gamma$ subunit mixture indicated the presence of both β_1 and β_2 in our preparation. We observed major fragments of 1730.4 and 2754 daltons, which correspond to N-acetylated β_1 -(2–15) (expected M_r of 1729) and N-acetylated β_2 -(2–23) (expected M_r of 2753). A minor peak of 2726.8 daltons was also observed, which could either correspond to N-acetylated β_1 -(2–23) (expected M_r of 2726) or an incomplete digestion fragment N-acetylated β_1 -(2–23) (expected M_r of 2729). The expected masses of labeled fragments were calculated from the mass of the β subunit fragment plus 965 daltons for the Lys C cleavage product of iodo-pHBDAP-Q (iodo-pHBDAP-Q-(11–14)). Thus, predicted labeled fragments from β_1 in the observed size range would have masses of 4658 (302–337), 5071 (302–341), 7011 (281–337), and 7424 (281–341), while those from β_2 would be 4944 (302–337) and 5057 (302–341). The difference in predicted fragment sizes for β_1 and β_2 is due to the arginine at position 280 in β_2 while β_1 has a cleavable lysine. All other fragments overlapping the carboxyl-terminal 23-kDa tryptic fragment would be larger than 8.9 kDa.

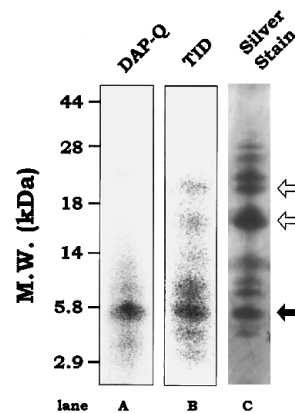


FIG. 3. Partial Lys C digest of purified $\beta\gamma$ labeled with [^{125}I]pHBDAP-Q. Purified $\beta\gamma$ subunit (14 μM) was photolyzed in the presence of [^{125}I]pHBDAP-Q (A and C, 3 μM , ~73 Ci/mmol) or [^{125}I]TID (B, 1 μM), and the products were electrophoresed on 12% SDS-PAGE. The cross-linked β subunit was excised, and the gel slices were loaded onto a 16% Tricine gel in the presence of Lys C as described under "Materials and Methods." The polyacrylamide gel was either fixed and exposed to a PhosphorImage screen (A and B) or silver-stained (C). The filled arrow indicates the ~6-kDa fragment labeled with DAP-Q, and the open arrows indicate two large fragments, which were not labeled with DAP-Q (see text). The data are representative of three separate experiments.

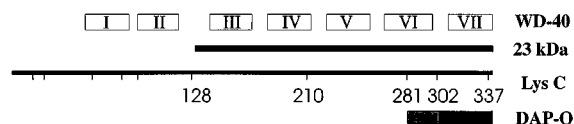


FIG. 4. Linear model of the β_1 subunit showing cleavage sites and the location of the DAP-Q labeling site. A linear model of β_1 subunit is shown indicating the 23-kDa carboxyl-terminal tryptic digestion fragment. Within this fragment the Lys C cut sites are shown by residue number following the cut. WD-40 repeat regions are indicated by the numbered boxes. The location of the DAP-Q labeling site is highlighted with the black region being the most likely site while the gray region cannot be absolutely excluded.

approximately 11.5 kDa from the β_2 subunit, which is not observed, it is likely that both subunits are labeled within residues 302–341 (black in Fig. 4). However, the ~6-kDa apparent molecular mass of the radiolabeled fragment is consistent also with cross-linking to β_1 in the region of 281–301 provided that cleavage at Lys³⁰¹ does not occur.

The absence of labeling within β_1 -(128–281) or β_2 -(128–301) is further supported by the major non-labeled fragments of approximately 16.5 and 19 kDa on the silver stain (Fig. 3C, open arrows). These are similar to the expected masses of 16,671 of β_1 -(128–280) and 19,077 of β_2 -(128–301). These partial Lys C digest fragments would be generated if cleavage did not occur at Lys²⁰⁹, which may be buried in the fourth WD-40 domain.

In summary, trypsin digest data indicate that the only sites of β subunit labeling by DAP-Q are within the carboxyl-terminal region of the β subunit. Lys C digestion shows that the majority of label migrated at ~6 kDa. Therefore, the radiolabeled Lys C fragment corresponds to a site within residues 302–341 of either β_1 or β_2 or possibly 281–301 of β_1 (Fig. 4). The region of DAP-Q labeling includes WD-40 repeat 7 and the connecting loop to 6 and possibly repeat 6 itself (Fig. 4). Interestingly, the DAP-Q binding site on β overlaps with the γ binding site on β , which has been shown to include portions in the carboxyl-terminal half of β (42–44).

In contrast to the limited region of contact with the receptor peptide, the membrane contact sites on β subunit appear to be much more extensive. They encompass both the amino- and carboxyl-terminal tryptic digest fragments, and within these

major fragments several small Lys C fragments are labeled. Further mapping will be required to define the details of membrane association as has been done for the Torpedo nicotinic acetylcholine receptor (40).

Functional data support DAP-Q as an appropriate tool for identifying receptor interaction sites on the β subunit. Our previous results showed that $\beta\gamma$ was required for peptide stimulation of α_o GTPase activity (12), just as is true for receptor activation of purified α subunits of G proteins (45). In addition to the site on β described in this report, we have shown that DAP-Q labels the amino terminus of α_o (12). This same NH₂-terminal region on α has been shown to bind to mastoparan (46) and to disrupt rhodopsin-transducin interactions (9). Although the regions on the β subunit that bind to α are not well defined, Neer and colleagues (47, 48) have shown that residues 204 and 271 in the carboxyl-terminal 23-kDa fragment of β_1 can be chemically cross-linked to the α subunit.

The sequences of β subunits are highly conserved and display approximately 83% amino acid identity among the known subtypes (49). In fact, within the region 281–341 only 8 amino acids are different among β_1 , β_2 , and β_4 (the most abundant subtypes). Are these few differences sufficient for somatostatin and muscarinic receptors to recognize different β subunits (21)? As noted above, DAP-Q and the γ subunit bind to overlapping regions on the β subunit. Thus, it is possible that the γ subunit could provide an additional level of specificity to the α_2 AR-G protein interaction. In accordance with this hypothesis, somatostatin receptors, muscarinic receptors, and rhodopsin all distinguish between G proteins composed of different γ subunits (22). Unlike the β subunits, γ subunits display great sequence diversity with only 30% homology between the known subtypes (49).

This report is the first to define regions of the β subunit that interact with a receptor-derived G protein activator and a membrane-associated photolabel. Based on these data and that of many other groups, it is likely that the binding site for receptor involves interactions with α , β , and γ subunits with the carboxyl terminus of β playing a significant role.

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REFERENCES

1. Sternweis, P. C. (1994) *Curr. Opin. Cell Biol.* **6**, 198–203
2. Kobilka, B. K., Matsui, H., Kobilka, T. S., Yang Feng, T. L., Francke, U., Caron, M., Lefkowitz, R. J., and Regan, J. W. (1987) *Science* **238**, 650–656
3. Strader, C. D., Dixon, R. A. F., Cheung, A. H., Candelore, M. R., Blake, A. D., and Sigal, I. S. (1987) *J. Biol. Chem.* **262**, 16439–16443
4. O'Dowd, B. F., Hnatowich, M., Regan, J. W., Leader, W. M., Caron, M. G., and Lefkowitz, R. J. (1988) *J. Biol. Chem.* **263**, 15985–15992
5. Dalman, H. M., and Neubig, R. R. (1991) *J. Biol. Chem.* **266**, 11025–11029
6. Palm, D., Munch, G., Dees, C., and Hekman, M. (1989) *FEBS Lett.* **254**, 89–93
7. Konig, B., Arendt, A., McDowell, J. H., Kahlert, M., Hargrave, P. A., and Hofmann, K. P. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 6878–6882
8. Ikezu, T., Okamoto, T., Ogata, E., and Nishimoto, I. (1992) *FEBS Lett.* **311**, 29–32
9. Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B., and Hofmann, K. P. (1988) *Science* **241**, 832–835
10. Wade, S. M., Dalman, H. M., Yang, S., and Neubig, R. R. (1994) *Mol. Pharmacol.* **45**, 1191–1197
11. Taylor, J. M., Jacob Mosier, G. G., Lawton, R. G., and Neubig, R. R. (1994) *Peptides* **15**, 829–834
12. Taylor, J. M., Jacob Mosier, G. G., Lawton, R. G., Remmers, A. E., and Neubig, R. R. (1994) *J. Biol. Chem.* **269**, 27618–27624
13. Sullivan, K. A., Miller, R. T., Masters, S. B., Beiderman, B., Heideman, W., and Bourne, H. R. (1987) *Nature* **330**, 758–760
14. Gutowski, S., Smrcka, A., Nowak, L., Wu, D., Simon, M., and Sternweis, P. (1991) *J. Biol. Chem.* **266**, 20519–20524
15. Palm, I. D., Munch, G., Malek, D., Dees, C., and Hekman, M. (1990) *FEBS Lett.* **261**, 294–298
16. Shenker, A., Goldsmith, P., Unson, C. G., and Spiegel, A. M. (1991) *J. Biol. Chem.* **266**, 802–808
17. Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G., and Spiegel, A. M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 7809–7813
18. Sternweis, P. C. (1986) *J. Biol. Chem.* **261**, 631–637
19. Hethier, H., Frohlich, M., Dees, C., Baumann, M., Haring, M., Gierschik, P., Schilta, E., Vaz, W. L. C., Hekman, M., and Helmreich, E. J. (1992) *Eur. J. Biochem.* **204**, 1169–1181
20. Phillips, W. J., and Cerione, R. A. (1992) *J. Biol. Chem.* **267**, 17032–17039
21. Kleuss, C., Scherubel, H., Hescheler, J., Shultz, G., and Wittig, B. (1992) *Nature* **358**, 424–426
22. Kleuss, C., Scherubel, H., Hescheler, J., Schultz, G., and Wittig, B. (1993) *Science* **259**, 832–834
23. Kisselev, O., and Gautam, N. (1993) *J. Biol. Chem.* **268**, 24519–24522
24. Kisselev, O. G., Ermolaeva, M. V., and Gautam, N. (1994) *J. Biol. Chem.* **269**, 21399–21402
25. Neer, E. J., Schmidt, C. J., Nambudripad, R., and Smith, T. F. (1994) *Nature* **371**, 297–300
26. Jacob Mosier, G., and Lawton, R. G. (1995) *J. Org. Chem.* **60**, 6953–6958
27. Roth, J. (1975) *Methods Enzymol.* **37**, 223–233
28. McConahey, P. J., and Dixon, F. J. (1980) *Methods Enzymol.* **70**, 210–213
29. Sternweis, P. C., and Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813
30. Kim, M. H., and Neubig, R. R. (1987) *Biochemistry* **26**, 3664–3672
31. Schaffner, W., and Weissmann, C. (1973) *Anal. Biochem.* **56**, 502–514
32. Roof, D. J., Applebury, M. L., and Sternweis, P. C. (1985) *J. Biol. Chem.* **260**, 16242–16249
33. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M., and Gilman, A. G. (1984) *J. Biol. Chem.* **259**, 3560–3567
34. Kwon, G., Remmers, A. E., Datta, S., and Neubig, R. R. (1993) *Biochemistry* **32**, 2401–2408
35. Thomas, T. C., Sladek, T., Yi, F., Smith, T., and Neer, E. J. (1993) *Biochemistry* **32**, 8628–8635
36. Cleveland, D. W. (1983) *Methods Enzymol.* **96**, 222–229
37. Laemmli, U. K. (1970) *Nature* **227**, 680–685
38. Schagger, H., and Von Jagow, G. (1987) *Anal. Biochem.* **166**, 368–379
39. Frielle, T., and Curthoys, N. P. (1983) *Biochemistry* **22**, 5709–5714
40. Blanton, M. P., and Cohen, J. B. (1994) *Biochemistry* **33**, 2859–2872
41. Simonds, W. F., Butrynski, J. E., Gautam, N., Unson, C. G., and Spiegel, A. M. (1991) *J. Biol. Chem.* **266**, 5363–5366
42. Garritsen, A., and Simmonds, W. F. (1994) *J. Biol. Chem.* **269**, 24418–24423
43. Katz, A., and Simon, M. I. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 1998–2002
44. Yamauchi, J., Kaziro, Y., and Itoh, H. (1995) *Biochem. Biophys. Res. Commun.* **214**, 694–700
45. Kelleher, D. J., and Johnson, G. L. (1988) *Mol. Pharmacol.* **34**, 452–460
46. Higashijima, T., and Ross, E. M. (1991) *J. Biol. Chem.* **266**, 12655–12661
47. Yi, F., Denker, B. M., and Neer, E. J. (1991) *J. Biol. Chem.* **266**, 3900–3906
48. Garcia-Higuera, I., Thomas, T. C., Yi, F., and Neer, E. J. (1995) *J. Biol. Chem.* **271**, 528–535
49. Spiegel, A. M., Jones, T. L. Z., Simonds, W. F., and Weinstein, L. S. (1994) *G proteins*, p. 77, R. G. Landes Company, Austin, TX