Journal of Cellular Biochemistry 47:352–358 (1991)

The NH_2-Terminal α Subunit Attenuator Domain Confers Regulation of G Protein Activation by βγ Complexes

N. Dhanasekaran, Shoji Osawa, and Gary L. Johnson

Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206 (N.D., S.O., G.L.J.); Department of Pharmacology, University of Colorado Medical School, Denver, Colorado 80226 (G.L.J.)

Abstract G_s and G_i, respectively, activate and inhibit the enzyme adenylyl cyclase. Regulation of adenylyl cyclase by the heterotrimeric G_s and G_i proteins requires the dissociation of GDP and binding of GTP to the α_s or α_i subunit. The βγ subunit complex of G_s and G_i functions, in part, to inhibit GDP dissociation and α subunit activation by GTP. Multiple β and γ polypeptides are expressed in different cell types, but the functional significance for this heterogeneity is unclear. The βγ complex from retinal rod outer segments ($\beta\gamma_t$) has been shown to discriminate between α_i and α_s subunits (Helman et al: Eur J Biochem 169:431–439, 1987). $\beta\gamma_t$ efficiently interacts with α_i-like G protein subunits, but poorly recognizes the α_s subunit. $\beta\gamma_t$ was, therefore, used to define regions of the α_i subunit polypeptide that conferred selective regulation compared to the α_s polypeptide. A series of α subunit chimeras having NH_2-terminal α_i and COOH-terminal α_s sequences were characterized for their regulation by $\beta\gamma_t$, measured by the kinetics of GTPγS activation of adenylyl cyclase. A 122 amino acid NH_2-terminal region of the α_i polypeptide encoded within an α/α_s chimera was sufficient for $\beta\gamma_t$ to discriminate the chimera from α_s. A shorter 54 amino acid α_i sequence substituted for the corresponding NH_2-terminal region of α_s was insufficient to support the α_i-like interaction with $\beta\gamma_t$. The findings are consistent with our previous observation (Osawa et al: Cell 63:697–706, 1990) that a region in the NH_2-terminal moiety functions as an attenuator domain controlling GDP dissociation and GTP activation of the α subunit polypeptide and that the attenuator domain is involved in functional recognition and regulation by βγ complexes.

Key words: G_i, G_s, chimeras, adenylyl cyclase

INTRODUCTION

The receptor-coupled G proteins are heterotrimers composed of α, β, and γ subunits [1,2]. The α subunit binds GDP and GTP, $\alpha\cdot_{GDP}$ being inactive and $\alpha\cdot_{GTP}$ active in their ability to regulate effector enzymes and ion channels. The β and γ subunits are tightly associated and regulate the α subunit activation state. One regulatory function of the βγ complex is its absolute requirement for efficient receptor-catalyzed GDP dissociation from the α subunit [3,4]. A second function of βγ is its attenuation of GDP dissociation and subsequent GTP activation of the α subunit in the absence of activated receptor [5].

Multiple β and γ subunits have now been identified by cDNA cloning and isolation of polypeptides [6–16]. Based on rather limited experimental evidence, different βγ complexes are interchangeable in their ability to reconstitute rhodopsin-catalyzed GDP/GTP exchange with α_i or α_t [17]. Also, βγ complexes purified from G_i and G_s appear to be interchangeable in reconstituting receptor coupling of α subunits and regulation of guanine nucleotide exchange [18–20]. However, $\beta\gamma_t$ discriminates between α_i and α_s, where $\beta\gamma_t$ efficiently recognizes α_i compared with its relatively poor recognition of α_s [18–20]. Cumulatively, the findings indicate that mixtures of brain βγ complexes equally recognize α_i-like

N. Dhanasekaran's present address is Department of Pharmacology, University of Wisconsin Medical School, Madison, WI 53706.

Shoji Osawa's present address is Department of Cell Biology and Anatomy, University of North Carolina Medical School, Chapel Hill, NC 27579.

Received July 5, 1991; accepted July 28, 1991.

Address reprint requests to Gary L. Johnson, Division of Basic Sciences, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

© 1991 Wiley-Liss, Inc.

(α_i, α_o, and α_t) and α_s polypeptides, whereas $\beta\gamma_t$ discriminates for α_i-like polypeptides relative to α_s.

The discrimination of α_s and α_i by $\beta\gamma_t$ must be related to differences in the primary amino acid sequences between the two α subunit polypeptides. The functional recognition domains for $\beta\gamma$ complexes within the α subunit primary sequence remains poorly defined. Proteolysis experiments with GTPγS or Gpp(NH)p activated G proteins have shown that removal of approximately 20 amino acids from the α subunit NH_2-terminus inhibits subsequent $\beta\gamma$ interaction with the truncated α chain polypeptide [21,22]. This finding has been interpreted to indicate that the first 20 or so residues of the α subunit NH_2-terminus are either part of the binding domain for $\beta\gamma$ complexes [21,22] or regulate $\beta\gamma$ interactions with the α chain polypeptide [23]. Little direct functional analysis beyond the proteolysis studies has been reported defining the α subunit sequences controlling $\beta\gamma$ complex interactions.

Previously, we characterized several α_i/α_s chimeras where different regions of the α_s primary sequence had been substituted with the corresponding region of α_{i2} [24,25]. Among these chimeras were several that had different lengths of the α_s NH_2-terminus substituted with the corresponding regions of the α_{i2} primary sequence. All of the NH_2-terminal α_i/α_s chimeras were capable of activating adenylyl cyclase. In this report, we have reconstituted NH_2-terminal α_i/α_s chimeras with $\beta\gamma_t$ isolated from bovine retina rod outer segments and assayed the effect of $\beta\gamma_t$ on the rate of GTPγS-stimulation of adenylyl cyclase activity. The use of G protein α_i/α_s chimeras uniquely utilizes the ability of $\beta\gamma_t$ to discriminate α_i-like polypeptides from α_s to map the functional $\beta\gamma$ recognition domain within the α subunit NH_2-terminus.

METHODS

The construction, DNA sequencing, and characterization of the $\alpha_{i(54)/s}$, $\alpha_{i(122)/s}$, and $\alpha_{i/s(Bam)}$ chimeras has been previously described [24,25]. The phenotypic properties of mutation of Gln227→Leu in the α_s polypeptide, referred to as α_sQ227L, has also been previously defined [24,26,27]. The wild-type rat G protein α_s subunit, each of the chimeric $\alpha_{i/s}$ and α_sQ227L cDNAs inserted in the pCW1 expression plasmid were transiently expressed in COS-1 cells using the DEAE-dextran transfection procedure [24,28]. Cells were used for measurement of intracellular cAMP levels or the preparation of crude plasma membranes 65 h after transfection. For cAMP measurements transfected COS-1 cells in 60 mm petri dishes were incubated for 10 min at room temperature in the presence of 500 μM methyl isobutylxanthine. Cellular cAMP was then extracted with 2 ml ice-cold 65% ethanol and lyophilized. Cyclic AMP was measured using the radioimmunoassay kit from Amersham Corp. according to the manufacturer's directions [29]. We have previously characterized this assay in transfected COS-1 cells to be an accurate measurement of cAMP synthesis and a valid assay of adenylyl cyclase activity in intact cells [24,25,29].

For preparation of membranes, cells were washed three times in ice-cold phosphate buffered saline, pH 7.4. Cells were then resuspended in ice-cold 20 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 1 mM EDTA and broken by 30–50 strokes of a Dounce homogenizer. Cell breakage was greater than 99% as determined by trypan blue exclusion and nuclei were removed by centrifugation at 2,000 x g for 10 min. The supernatant from the low speed spin was then centrifuged at 50,000 x g for 30 min and the pellet resuspended and used as a crude membrane preparation for assay of adenylyl cyclase and immunoblotting analysis.

Rod outer segments were isolated and transducin (G_t) was purified as described previously [30]. The $\beta\gamma_t$ complex was then purified and resolved from α_t on a blue-Sepharose column [4]. The concentration of $\beta\gamma_t$ was estimated by Bradford protein assay [31], relative to bovine serum albumin standard, which yielded values similar to that derived from Coomassie staining of electrophoresed bands of α_t and β_t [4]. For denaturation of $\beta\gamma_t$, samples were boiled for 10 min and then cooled on ice prior to reconstitution with COS cell membranes. For reconstitution, 0.1, 1.0, or 10 μg native or denatured $\beta\gamma_t$ was mixed with 60 μg COS cell membrane protein in 20 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 1 mM EDTA for 20 min on ice. The adenylyl cyclase reaction was initiated by addition of the reconstituted membranes to a reaction mixture pre-equilibrated at 30°C and 50 μg COS cell membrane protein aliquots removed at appropriate times and placed in 1 ml 1% sodium dodecyl sulfate to stop the reaction. Adenylyl cyclase

activity was measured by the conversion of [α-^{32}P]ATP to [^{32}P]cAMP [29].

Membranes from transfected COS cells were also used for α_s polypeptide immunoblotting. Seventy micrograms of membrane protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% acrylamide), transferred to nitrocellulose, and probed with antibodies specific for the carboxy-terminus of α_s [25]. The mutant and wild-type α_s polypeptides were all expressed at levels 4–5 times greater than the endogenous α_s polypeptide in the COS cell membranes [24].

RESULTS AND DISCUSSION

We have used three chimeras in which different lengths of the α_s NH_2-terminus were substituted with the corresponding region of α_{i2} (Fig. 1A) in an attempt to define domains within the α subunit primary sequence responsible for selective interaction with $\beta\gamma_t$ complexes. The chimera with the shortest α_{i2} sequence at the NH_2-terminus is $\alpha_{i(54)/s}$, which has the first 61 amino acids of α_s replaced by the first 54 amino acids of α_{i2}. There are seven more amino acids in α_s than in α_{i2} within this region of the NH_2-terminus, and 16 α_{i2} residues are nonconserved compared with the α_s sequence. The second chimera is $\alpha_{i(122)/s}$, which substitutes the first 144 residues of α_s with the corresponding 122 residues of α_{i2}. There are 38 nonconserved residues between α_{i2} and α_s within the junctions of the $\alpha_{i(54)/s}$ and $\alpha_{i(122)/s}$ chimeras. The third chimera is $\alpha_{i/s(Bam)}$ which encodes the first 212 residues of α_{i2} and the COOH-terminal 160 residues of α_s [25,32]. Within the NH_2-terminal moiety encoding α_{i2} of the $\alpha_{i/s(Bam)}$ chimera, approximately 35% of the amino acids are nonconserved relative to the corresponding region of the α_s polypeptide.

The $\alpha_{i(54)/s}$, $\alpha_{i(122)/s}$, and $\alpha_{i/s(Bam)}$ chimeras previously were shown to function as α_s polypeptides capable of activating adenylyl cyclase [24,25]. When transiently expressed in COS cells, each of the $\alpha_{i/s}$ chimeras stimulate cAMP synthesis (Fig. 1B). The $\alpha_{i(122)/s}$ and $\alpha_{i/s(Bam)}$ chimeras stimulate cAMP synthesis to levels similar to those observed with expression of the wild-type α_s polypeptide. In contrast, the $\alpha_{i(54)/s}$ chimera stimulates markedly higher cAMP synthesis when expressed in COS cells relative to the wild-type α_s polypeptide. We have demonstrated that the activated state of the $\alpha_{i(54)/s}$ chimera results from the altered regulation of GDP dissociation and an enhanced rate of GTP activation [25]. Mutation of the α_s NH_2-terminus by substitution of the corresponding region of α_{i2} was shown to alter the properties of the GDP/GTP binding domain independent of the GTPase activity in-

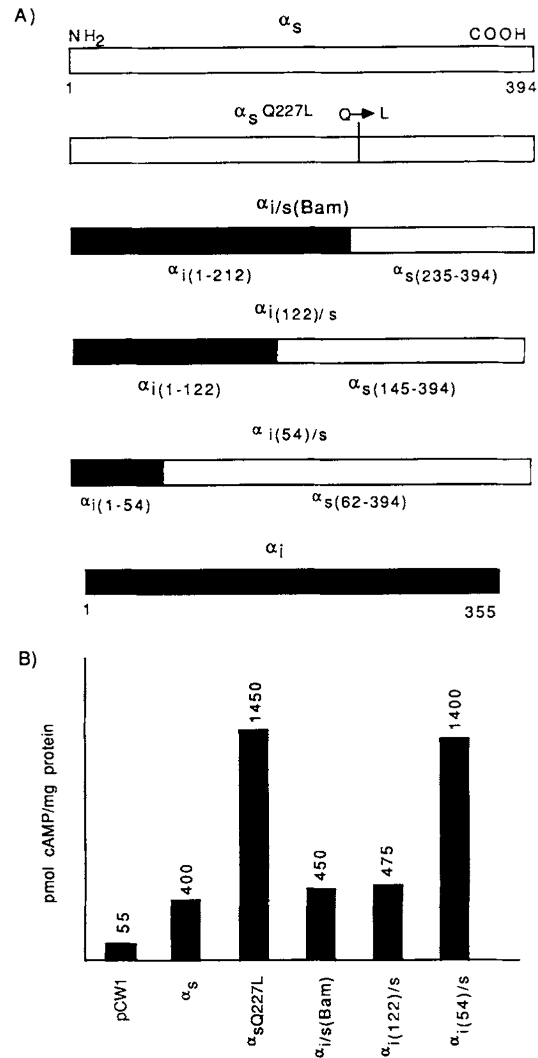

Fig. 1. Effect of mutant α_s polypeptides on cAMP levels in transfected COS cells. A: Map of the 394 and 355 amino acid α_s and α_i polypeptides, the α_sQ227L mutant and α_i/α_s chimeras. B: Intracellular cAMP accumulations in COS cells expressing the different $\alpha_{i/s}$ chimeras and GTPase inhibited α_sQ227L mutant. Cyclic AMP levels were measured 65 h after transfection in the presence of the phosphodiesterase inhibitor, methyl isobutylxanthine (500 μM), as described in Methods. The wild-type α_s, α_sQ227L, $\alpha_{i/s(Bam)}$, $\alpha_{i(122)/s}$, and $\alpha_{i(54)/s}$ polypeptides were similarly over-expressed 4–5 times greater than the level of the endogenous α_s subunit [24]. Values represent the mean of triplicate determinations that varied by less than 10% and are representative of two independent experiments. Cyclic AMP values (picomoles/mg cellular protein) are shown above the bar for each construct.

trinsic to the α chain polypeptide [25]. The activated character of the $\alpha_{i(54)/s}$ chimera is, however, similar to that observed with the α_sQ227L mutant (Fig. 1B), whose GTPase activity is strongly inhibited [24,26,27]. In fact, the $\alpha_{i(54)/s}$ mutant is additive with the α_sQ227L mutation when both are placed within the same polypeptide ($\alpha_{i(54)/s\text{-}Q227L}$). Because $\alpha_{i(54)/s}$ enhances the rate of activation and α_sQ227L inhibits GTPase activity, the two mutations independently inhibit the two rate-limiting steps in α chain activation [24].

The difference in activation state of the $\alpha_{i(54)/s}$ and $\alpha_{i(122)/s}$ polypeptides has been used to define the NH_2-terminal region within the first 122 amino acids as an attenuator control domain regulating GDP dissociation and subsequent GTP activation [24,25]. The regulatory properties assigned to the α subunit attenuator control domain were shown to be disrupted in the $\alpha_{i(54)/s}$ chimera, but normal in the $\alpha_{i(122)/s}$ and $\alpha_{i/s(Bam)}$ polypeptides. The properties of the α subunit attenuator control domain overlap with those that have been described for the $\beta\gamma$ complex, suggesting that amino acids within the NH_2-terminal 122 residues within the α_i domain of the $\alpha_{i(122)/s}$ polypeptide are required for normal $\beta\gamma$ interaction with the α subunit. Disruption of the attenuator control domain sequence in the $\alpha_{i(54)/s}$ chimera causes a loss in the control of GDP dissociation and α_s activation by GTP, a function assigned to the $\beta\gamma$ complex. We have used the differential discrimination of $\beta\gamma_t$ for α_i-like polypeptides relative to α_s to directly test the disruption of $\beta\gamma$ control of α_s activation by mutation of the attenuator control domain. Since $\alpha_{i(54)/s}$, $\alpha_{i(122)/s}$, and $\alpha_{i/s(Bam)}$, respectively, have 54, 122, and 212 amino acids encoding the α_{i2} NH_2-terminal primary sequence, it was possible to test which chimera encoded sufficient α_i sequence for selective discrimination relative to α_s polypeptides.

In order to assay the ability of the different chimeras to be differentially regulated by $\beta\gamma_t$, as measured by GTPγS stimulation of adenylyl cyclase, we took advantage of the fact that reconstitution of $\beta\gamma$ complexes with membrane preparations will diminish the rate of activation of adenylyl cyclase by guanine nucleotides [5]. The time required to reach maximal GTPγS activated adenylyl cyclase activity will be increased when α_s polypeptides are associated with $\beta\gamma$ complexes, because $\beta\gamma$ inhibits GDP dissociation which must occur before GTPγS can bind [1–4]. Adding $\beta\gamma$ complexes to the membrane assay system thus prolongs the time required to reach maximal α_s activation [1,2,5]. The inhiibition of α_s activation by $\beta\gamma$ complexes can be easily measured by the time or lag required for obtaining maximal adenylyl cyclase activity. The ability of $\beta\gamma_t$ to discriminate between α_i and α_s polypeptides was used to determine which chimeras were more sensitive to inhibition by reconstitution of $\beta\gamma_t$ complexes with the COS cell membranes.

The effect of increasing concentrations of $\beta\gamma_t$ on the rate of GTPγS-stimulated adenylyl cyclase activity in COS cell membranes expressing the wild-type, chimeric, and mutant α_s polypeptides is shown in Figure 2. For each α_s construct the time course for GTPγS stimulation of adenylyl cyclase activity was determined in the presence of 0.1, 1.0, and 10 μg of purified native or denatured $\beta\gamma_t$. The control lag time to reach the maximal rate of GTPγS-stimulated cyclic AMP synthesis ranged from 4.6–5.8 min in different preparations of membranes from COS cells expressing the wild-type α_s, $\alpha_{i(122)/s}$, $\alpha_{i/s(Bam)}$, and α_sQ227L polypeptides. The lag time for GTPγS stimulation in membranes expressing $\alpha_{i(54)/s}$ was reduced to 2.6 min, indicative of the activated nature of the $\alpha_{i(54)/s}$ chimera, which is related to its enhanced rate of GTP activation relative to the wild-type α_s polypeptide. With 10 μg native $\beta\gamma_t$ added to the adenylyl cyclase assay reaction mixture relative to controls with denatured $\beta\gamma_t$, the time required to reach the maximal GTPγS-stimulated rate of cAMP synthesis was similarly increased by approximately 1.4–1.6 min for membranes prepared from COS cells overexpressing wild-type α_s, $\alpha_{i(54)/s}$, $\alpha_{i(122)/s}$, $\alpha_{i/s(Bam)}$, and α_sQ227L. Thus, at high $\beta\gamma_t$ levels in the reconstituted membranes, the lag time required for maximal GTPγS stimulation was similar for all of the chimeras, suggesting that this was a sufficiently high concentration of $\beta\gamma_t$ for interaction of $\beta\gamma_t$ with the α_s polypeptide, as well as the α_i/α_s chimeras as previously demonstrated [18–20].

However, at a 10-fold lower concentration of $\beta\gamma_t$ selective differences in the ability of $\beta\gamma_t$ to influence the lag time for GTPγS activation of adenylyl cyclase was detected. Thus, 1 μg $\beta\gamma_t$ appears to be capable of selectively discriminating between α_i and α_s sequences within the α_i/α_s chimeras. Addition of 1 μg $\beta\gamma_t$ increased the lag time for GTPγS activation of adenylyl cyclase for the membranes expressing the $\alpha_{i(122)/s}$ and

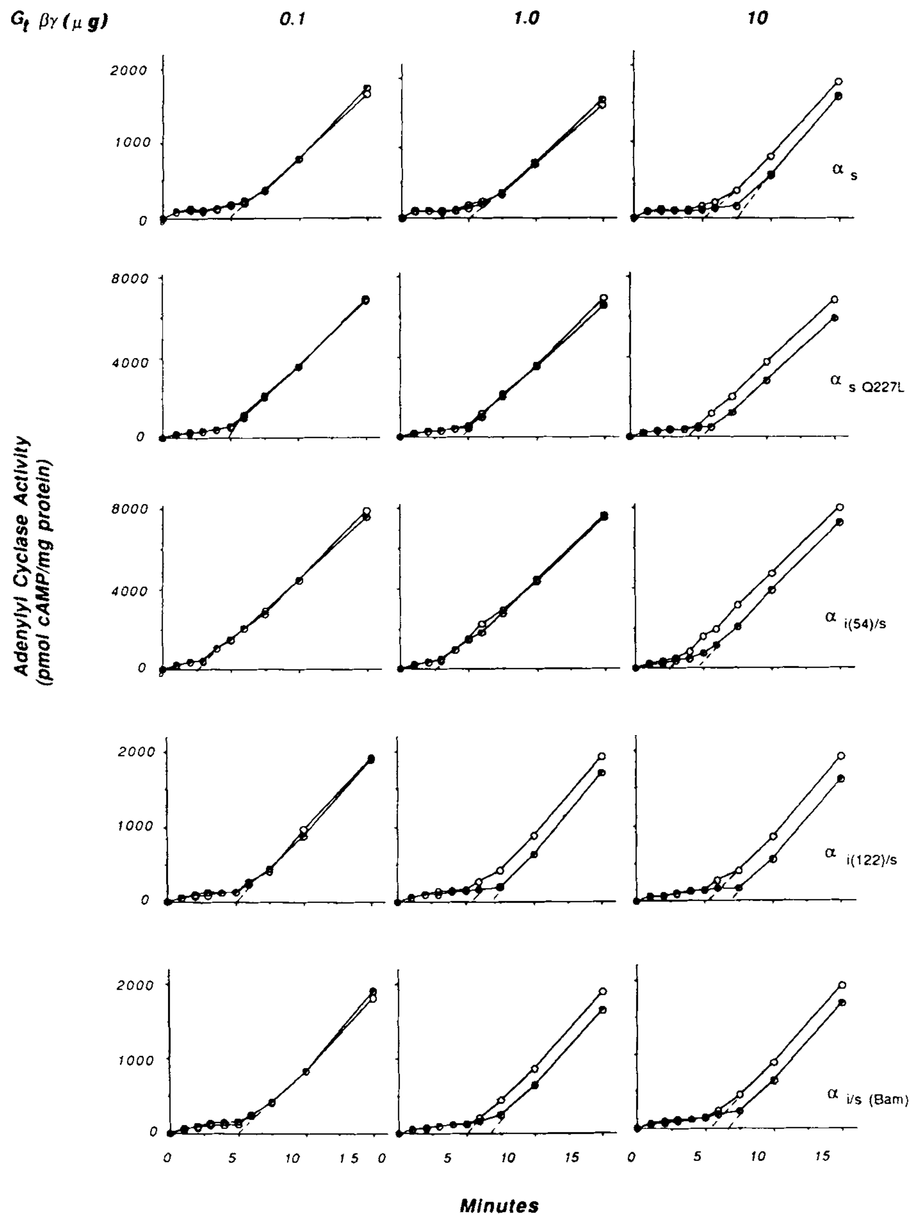

Fig. 2. Time course of GTPγS-stimulated adenylyl cyclase activity in COS membranes expressing α_i/α_s chimeras in the presence of $\beta\gamma_t$. Purified $\beta\gamma_t$ was reconstituted with COS cell membranes overexpressing α_s, α_sQ227L, $\alpha_{i(54)/s}$, $\alpha_{i(122)/s}$, or $\alpha_{i/s(Bam)}$ polypeptides as described in Methods. For each membrane preparation 0.1, 1, or 10 μg native (dark circles) or denatured (open circles) $\beta\gamma_t$ was used for reconstitution. The time course of GTPγS-stimulated adenylyl cyclase activity was determined for COS cell membranes expressing each construct, and the time required to reach V_{max} was estimated by extrapolation of the maximal rate to the time line (---). The estimated lag times to achieve GTPγS-stimulated V_{max} is summarized in Table I. The time course was repeated with each $\beta\gamma_t$ concentration with COS membranes from three independent transfections for each construct. The results shown are from one complete set of transfections and are representative of the findings for the three independent experiments. Only the $\alpha_{i(122)/s}$ and $\alpha_{i/s(Bam)}$ constructs showed a shift in the lag time required to reach V_{max} in the presence of 1.0 μg native $\beta\gamma_t$, which was reproducible in all three experiments. The shift in the time to reach V_{max} at 10 μg native $\beta\gamma_t$ was similar in all three experiments for COS cell membranes expressing the designated α_s constructs.

$\alpha_{i/s(Bam)}$ chimeras. The lag time to achieve maximal adenylyl cyclase activation for the α_s, $\alpha_{i(54)/s}$, and α_sQ227L expressing membranes was unchanged in the presence of 1 μg $\beta\gamma_t$ relative to controls with denatured $\beta\gamma_t$ complex. This is in contrast to the addition of 10 μg $\beta\gamma_t$ in the reaction mixture, which influenced the lag time for GTPγS activation for all of the COS cell membranes tested expressing the wild-type and mutant α_s polypeptides. The estimated times required to reach maximal GTPγS-stimulated adenylyl cyclase activity in membranes expressing the wild-type and mutant α_s polypeptides is summarized in Table I. The results demonstrate that only the $\alpha_{i(122)/s}$ and $\alpha_{i/s(Bam)}$ chimeras encoded sufficient α_{i2} primary sequence to shift the potency to lower concentrations of $\beta\gamma_t$ to delay the rate of GTPγS activation; $\alpha_{i(54)/s}$ lacked sufficient α_{i2} NH_2-terminal sequence to allow selective regulation by $\beta\gamma_t$ relative to the α_s polypeptide. Thus, residues within the first 122 amino acids within the attenuator control domain of α_{i2} are required for the selective discrimination by $\beta\gamma_t$ of α_i-like polypeptides relative to α_s subunits. The findings indicate that the attenuator control domain is directly involved in $\beta\gamma$ interactions and the regulation of the GDP/GTP binding domain.

Several points must be addressed relating to the interpretation of the findings in Figure 2 and Table I. First, we have previously shown that neither the expression of α_i nor of $\beta\gamma$ complexes is changed in COS cells with expression of any other chimeras or α_sQ227L [24,25,29,33]. Second, prior treatment of the cells with pertussis toxin does not change the time course of GTPγS-stimulated adenylyl cyclase or cAMP accumulation resulting from expression of the chimeras [24,25]. Third, the expression of wild-type α_{i2} in COS cells does not influence the time course of GTPγS activation of adenylyl cyclase activity (not shown). These results indicate that the phenotypic effects of the chimeras are a function of their α_s-like properties and activation of adenylyl cyclase.

It should also be noted that the $\alpha_{i(54)/s}$ chimera and the α_sQ227L mutant are constitutively activated. The $\alpha_{i(54)/s}$ chimera has been shown to have a markedly diminished lag time for GTPγS activation, but normal GTPase activity [24,25,33]. In contrast, the major phenotypic property of the α_sQ227L mutant is an inhibited GTPase activity, but relatively normal lag time for GTPγS activation of adenylyl cyclase [24,26,27].

TABLE I. Lag Time Required to Reach Maximal GTPγS-Stimulated Adenylyl Cyclase Activity*

	Lag time (minutes) native/ denatured (Δ)		
$\beta\gamma_t$, μg	0.1	1.0	10
α_s	5.4/5.4 (0)	5/5 (0)	7.2/5.8 (1.4)
α_sQ227L	4.6/4.6 (0)	4.6/4.6 (0)	6.2/4.8 (1.4)
$\alpha_{i(54)/s}$	2.6/2.6 (0)	2.6/2.6 (0)	3.8/2.4 (1.4)
$\alpha_{i(122)/s}$	5.2/5.2 (0)	7/5.6 (1.4)	7.4/5.8 (1.6)
$\alpha_{i/s(Bam)}$	5.4/5.4 (0)	7/5.8 (1.2)	6.8/5.4 (1.4)

*Extrapolation of the GTPγS-stimulated adenylyl cyclase V_{max} measured in Figure 2 was used to estimate the time when maximal adenylyl cyclase activity was achieved. The data is presented as the lag time to reach maximal GTPγS-stimulated adenylyl cyclase activity in the presence of the indicated amount of native or denatured $\beta\gamma_t$ reconstituted with COS cell membranes as described in Methods. The values in parentheses are the difference (Δ) in time required to reach GTPγS-stimulated V_{max} in the presence of native versus denatured $\beta\gamma_t$. The data represent the findings from three independent experiments for each α_s construct, where the extrapolated values varied by less than 5% between each experiment.

Both show a shift in the lag time for GTPγS activation in the presence of 10 μg $\beta\gamma_t$, indicating that they are capable of interacting with $\beta\gamma$ complexes. This is consistent with their ability to efficiently couple to β-adrenergic receptors [24,26,27,33], a function that requires the association with $\beta\gamma$ complexes [1–5]. However, the $\alpha_{i(54)}$ sequence was shown to disrupt the ability of $\beta\gamma$ to mediate pertussis toxin-catalyzed ADP-ribosylation of chimeras also having the α_{i2} sequence at the COOH-terminus of α_s polypeptides [24]. This previous result combined with the data in Figure 2 demonstrates that the first 54 residues of α_{i2} are not sufficient to allow discrimination by $\beta\gamma_t$ relative to the α_s polypeptide. In contrast, both $\alpha_{i(122)/s}$ and $\alpha_{i/s(Bam)}$ showed similar shifts at 1 μg $\beta\gamma_t$ that were not observed with α_s, α_sQ227L, or $\alpha_{i(54)/s}$. Cumulatively, these results indicate that the discrimination of α_{i2} relative to α_s by $\beta\gamma_t$ requires more than the first 54 residues of α_{i2}, and that the first 122 residues are as effective as the NH_2-terminal 212 amino acids of α_{i2}. We do not intend to suggest that the 122 amino acid sequence at the α_{i2} NH_2-terminus is the only "domain" independent of other amino acids within the tertiary structure of the α subunit polypeptide that interacts with the $\beta\gamma$ complex. Rather, we propose that the NH_2-terminal 122 amino acid domain of α subunits has a major regulatory role in $\beta\gamma$

interactions and that mutation in this region has the potential to dramatically disrupt the regulation of α-chain activation (e.g., $\alpha_{i(54)/s}$ chimera).

Previously, studies that proteolytically removed the NH_2-terminal 20 amino acids of α_t or α_o demonstrated that βγ no longer interacted with the truncated α chain polypeptide [21,22], indicating that this region in the NH_2-terminus must indeed be an important domain for controlling βγ interactions. Previously, we reported the characterization of an additional $\alpha_{i/s}$ chimera that substituted the first 14 amino acids of α_s with the first 7 amino acids of α_{i2}, referred to as the $\alpha_{i(7)/s}$ chimera [24]. The $\alpha_{i(7)/s}$ chimera behaved similar to wild-type α_s and not $\alpha_{i(54)/s}$ in its activation of adenylyl cyclase, indicating that mutation of the immediate NH_2-terminal α_s residues was not sufficient to cause the activated phenotype characteristic of the $\alpha_{i(54)/s}$ chimera. The simplest interpretation of the proteolysis experiments and the phenotypes of the $\alpha_{i(7)/s}$, $\alpha_{i(54)/s}$, $\alpha_{i(122)/s}$, and $\alpha_{i/s(Bam)}$ chimeras is that residues near the extreme NH_2-terminus are required for binding of βγ complexes, but that discrimination by βγ complexes of α_{i2} and α_s requires additional amino acids within the first 122 amino acids of the α_{i2} polypeptide. For the different α subunit polypeptides whose sequence has been defined, this NH_2-terminal domain has regions that are quite variable in amino acid composition, suggesting these sequence differences may be involved in the discrimination of different βγ subunit complexes. Additional expression, mutation and reconstitution analysis will determine what sequence differences in this domain of different G protein α subunits are capable of encoding βγ subtype selectivity, providing an additional tier of regulation in receptor activation of G protein regulated effector systems.

ACKNOWLEDGMENTS

Supported by NIH grants GM 30324 and DK 37871 and the American Heart Association.

REFERENCES

1. Gilman AG: Annu Rev Biochem 56:615–649, 1987.
2. Johnson GL, Dhanasekaran N: Endoocr Rev 10:317–331, 1989.
3. Navon SE, Fung BK-K: J Biol Chem 262:15746–15751, 1987.
4. Kelleher DJ, Johnson GL: Mol Pharmacol 34:452–460, 1988.
5. Birnbaumer L, Abramowitz J, Brown AM: Biochim Biophys Acta 1031:163–224, 1990.
6. Sugimoto K, Toshihide N, Tanabe T, Takahashi H, Noda M, Minamino N, Kangawa K, Matsuo H, Hirose T, Ikmagama S, Numa S: FEBS Lett 191:235–240, 1985.
7. Gao B, Gilman AG, Robishaw JD: Proc Natl Acad Sci. USA 84:6122–6125, 1987.
8. Gao B, Mumby S, Gilman AG: J Biol Chem 262:17524–17527, 1987.
9. Fong HKW, Amatruda T, Birren BW, Simon MI: Proc Natl Acad Sci USA 84:3792–3796, 1987.
10. Gierschik P, Codina J, Simons C, Birnbaumer L, Spiegel A: Proc Natl Acad Sci USA 82:727–731, 1985.
11. Levine MA, Smallwood LM, Moen PT, Hellman LJ, and Ahn TG: Proc Natl Acad Sci USA 87:2329–2333, 1990.
12. Fong HKW, Hurley JB, Hopkins RS, Miake-Lye R, Johnson MS, Doolittle RF, Simon MI: Proc Natl Acad Sci. USA 83:2162–2166, 1986.
13. Amatruda T, Gautam N, Fong HKW, Northup JK, Simon MI: J Biol Chem 263:5008–5011, 1988.
14. Hurley JB, Fong HK, Teplow DB, Dreyer WJ, Simon MI: Proc Natl Acad Sci USA 81:6948–6952, 1984.
15. Robishaw JD, Kalman VK, Moomaw CR, Slaughter CA: J Biol Chem 264:15758–15761, 1989.
16. Hildebrandt JD, Codina J, Rosenthal W, Birnbaumer L, Neer EJ, Yamazaki A, Bitensky MW: J Biol Chem 260:14867–14872, 1985.
17. Kanaho Y, Tsai SC, Adamik R, Hewlett EL, Moss J, Vaughan M: J Biol Chem 259:7378–7381, 1984.
18. Cerione RA, Gierschik P, Staniszewski C, Benovic JL, Codina J, Somers R, Birnbaumer L, Speigel AM, Lefkowitz RJ, Caron MG: Biochemistry 26:1485–1491, 1987.
19. Heckman M, Holzhofer A, Gierschik P, Im MJ, Jakobs KH, Pfeuffer T, Helmreich EJM: Eur J Biochem 169: 431–439, 1987.
20. Casey PJ, Graziano MP, Gilman AG: Biochemistry 28: 611–616, 1989.
21. Navon SE, Fung BK-K: J Biol Chem 262:15746–15751, 1987.
22. Neer EJ, Pulsifer L, Wolf LG: J Biol Chem 263:8996–9000, 1988.
23. Masters SB, Stroud RM, Bourne HR: Protein Eng 1:47–54, 1986.
24. Osawa S, Dhanasekaran N, Woon CW, Johnson GL: Cell 63:697–706, 1990.
25. Osawa S, Heasley LE, Dhanasekaran N, Gupta SK, Woon CW, Berlot C, Johnson GL: Mol Cell Biol 10:2931–2940, 1990.
26. Masters SB, Miller TR, Chi M-H, Chang F-H, Beiderman B, Lopez NG, Bourne HR: J Biol Chem 264:15467–15474, 1989.
27. Graziano MP, Gilman AG: J Biol Chem 264:15475–15482, 1989.
28. Ausubel FM, Brent R, Kingston RE, Moore DD, Smith JA, Seidman JG, Struhl K: "Current Protocols in Molecular Biology." (New York: John Wiley and Sons), 1987.
29. Woon CW, Soparkar S, Heasley LE, Johnson GL; J Biol Chem 264:5687- 5693, 1988.
30. Wessling-Resnick M, Johnson GL: Biochemistry 26: 4316–4322, 1987.
31. Bradford MM: Anal Biochem 72:248–254, 1976.
32. Masters SB, Sullivan KA, Miller RT, Beiderman B, Lopez NG, Ramachandran J, Bourne HR: Science 241: 448–451, 1988.
33. Osawa S, Johnson GL: J Biol Chem 266:4673–4676, 1991.