Adenylyl Cyclase Type II Domains Involved in G$\beta\gamma$ Stimulation[†]

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ABSTRACT: Mammalian particulate adenylyl cyclases contain two transmembrane regions (M_1 and M_2) and two cytosolic domains (C_1 and C_2) forming the catalytic core. The cytosolic domains are subdivided into a highly conserved region (part a) and a region with lower similarity (part b). Hypothetical models exist that account for the mechanism by which Gα_s and forskolin stimulate mammalian adenylyl cyclase. In contrast, little is known about how G$\beta\gamma$ dimers regulate catalysis. The so-called QEHA region located in the C_{2a} domain of type II adenylyl cyclase has been proposed to represent a site of interaction. Here we show (i) that the QEHA region directly interacts with G$\beta\gamma$ but (ii) that it is of minor importance for the stimulation of type II adenylyl cyclase because it can be replaced by corresponding, nonidentical regions of other adenylyl cyclase isoforms without altering the stimulatory effect of G$\beta\gamma$ and (iii) that the C_{1b} region is necessary for G$\beta\gamma$ to exert a stimulatory effect on adenylyl cyclase type II as in a C_{1b} deletion mutant the G$\beta\gamma$ regulation was specifically impeded whereas the Gα_s- and forskolin-mediated stimulation was maintained.

Particulate mammalian adenylyl cyclases (ACs;[1] EC 4.6.1.1) represent a family of at least nine different isoforms (ACI to ACIX) that have been cloned, expressed, and characterized. All isoforms can be activated by the α-subunits of heterotrimeric stimulatory guanine nucleotide-binding proteins (Gα_s) and—with the exception of ACIX—also by the diterpene forskolin. They differ in their response to modulators such as the α-subunits of inhibitory G proteins (Gα_i), Ca^{2+}, Ca^{2+}/calmodulin, and G$\beta\gamma$ (complex of G protein β- and γ-subunits; refs *1* and *2*). Individual G protein α-subunits regulate different isoforms of ACs in an identical manner; i.e., Gα_s stimulates and Gα_i inhibits all isoforms that are sensitive to the regulator. In contrast, G$\beta\gamma$ complexes either activate (ACII, ACIV, and probably ACVII) or inhibit (ACI) the enzyme (*3*−*5*).

Mammalian particulate ACs are integral membrane proteins which comprise two large cytosolic domains (C_1 and C_2), each preceded by a set of six transmembrane spans designated M_1 and M_2 (Figure 3A; ref *6*). The C_1 and C_2 domains are subdivided by similarity in parts a and b, with C_{1a} and C_{2a} being well conserved. Linking C_{1a} to C_2 or mixing these domains after separate expression generates an ATP-cyclizing soluble activity that is regulated by Gα_s and forskolin (*7*−*9*). The crystal structure of soluble AC revealed interaction sites for Gα_s and gave insight into the mechanism by which Gα_s activates AC (*10*).

G$\beta\gamma$ is a conditional activator of ACII and conditional inhibitor of ACI; i.e., both modes of regulation are best observed in the presence of an activator like Gα_s. Little is known about domains that are involved in G$\beta\gamma$ regulation of ACII. The C-terminal half of ACII (II−M_2C_2) has been tentatively assigned as the site for activation by G$\beta\gamma$ (*4*) on the basis of data with a particulate chimera (I−M_1C_1 + II−M_2C_2). Deletion of both transmembrane spans M_1 and M_2 released the chimera from the membrane. This soluble chimera (I−C_{1a}−II−C_2) was not stimulated by G$\beta\gamma$ (*8*), pointing to the M_2 domain as an important determinant in G$\beta\gamma$ regulation. More recently, amino acids 956−982 of ACII (QEHA region) have been described to interact with the G$\beta\gamma$ complex (*11*, *12*). The QEHA region is located in the C_2 domain and thus within the region proposed by Tang and Gilman, but it has not been determined if the QEHA region really contributes to G$\beta\gamma$ stimulation.

In this study, we have mutated amino acids in the QEHA region of ACII. Surprisingly, none of these substitutions altered the stimulatory response of the AC mutant to G$\beta\gamma$, thereby excluding the QEHA region to be a major determinant in G$\beta\gamma$-mediated AC stimulation. We present data that assign to the C_{1b} subdomain an important role in ACII activation as revealed by in vitro binding of G$\beta\gamma$ to immobilized AC and an ACII deletion mutant.

MATERIALS AND METHODS

Generation of ACII Constructs.[2] The cDNA encoding ACII in pBluescript KS (Stratagene) was used to generate ACII−I_{QEHA} and ACII−III_{QEHA}. ACII−I_{QEHA} was generated according to the instructions of the GeneEditor System (Promega) with one mutagenic oligonucleotide. ACII−I_{QEHA} was then ligated to pFastBac1 (*Xho*I, *Kpn*I; Life Technolo-

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[1] Abbreviations: AC, adenylyl cyclase; BSA, bovine serum albumin; $C_{12}E_{10}$, polyoxyethylene 10-lauryl ether; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid, DTT, dithiothreitol; G protein, heterotrimeric guanine nucleotide-binding protein; PAG, polyacrylamide gel; PMSF, phenylmethanesulfonyl fluoride.

[2] Sequences of primers are available upon request.

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gies). ACII−III$_{QEHA}$ was generated by PCR and four-way ligation. A site for *Bsm*BI was intermittently introduced by PCR. Three fragments were amplified: the ACIII fragment using ACIII−cDNA as a template, and the ACII 5′-fragment and ACII 3′-fragment using ACII as a template. The ACIII fragment was digested with *Bsm*BI, the ACII 5′-fragment with *Bsp*EI and *Bsm*BI, and the ACII 3′-fragment with *Bsm*BI and *Spe*I. All fragments were ligated into ACII in pBluescript KS (*Bsp*EI, *Spe*I) to generate ACII−III$_{QEHA}$, which then was ligated to pFastBac1 (*Eco*RI, *Spe*I). The generation of constructs encoding N- and C-terminal halves of ACII (II−M_1C_1 and II−M_2C_2) has been described (*13*). II−M_1C_{1a} was generated by PCR of the II−C_{1a} region, followed by ligation into pQE30 (*Sph*I, *Hin*dIII). Then, the AC-coding fragment was released (*Apa*I, *Hin*dIII) to replace II−C_1 of II−M_1C_1 (*Apa*I, *Hin*dIII) in pFastBac1. For GST−AC fusion proteins parts of the ACII−cDNA were amplified by PCR. Fragments were cut with *Bgl*II and *Eco*RI (II−C_1, II−C_{1a}, II−C_2) or with *Bam*HI and *Eco*RI (II−C_{1b}) and ligated to pGEX-2TK (*Bam*HI, *Eco*RI; Pharmacia).

Expression of Proteins. Baculoviruses were generated from pFastBac1 constructs (Bac-to-Bac, Life Technologies). The expression of ACs and the preparation of membrane fractions have been described elsewhere (*13*). Cytosolic fractions were isolated by resuspending cells in lysis buffer (20 mM Na-Hepes, pH 8, 5 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1 mM DTT, 0.1 mM PMSF, 3.3 μg/mL leupeptin, 3.2 μg/mL soybean trypsin inhibitor, 2 μg/mL aprotinin) and subsequent nitrogen cavitation. The 50000*g* supernatant was flash frozen in liquid nitrogen and stored at −80 °C. Proteins in membrane and cytosolic fractions were quantified according to Bradford with BSA as standard (*14*).

GST−AC fusion proteins were expressed in *Escherichia coli*.

$G\alpha_s$ for AC costimulation was applied as the constitutively active mutant $G\alpha_sQ213L$ derived from bovine $G\alpha_{s-short}$ with a C-terminal histidine tag (*15*). $G\alpha_sQ213L$ was expressed in *E. coli* and purified (*16*).

Recombinant bovine $G\beta_1\gamma_2$, $G\beta_1H_6\gamma_2$, and $G\beta_1H_6\gamma_2$-C68S were expressed in Sf9 cells and purified (*17*).

Detection of Gβγ Binding to GST−AC Fusion Proteins. Bacteria expressing GST fusion proteins were resuspended in buffer A (20 mM Na-Hepes, pH 8, 2 mM EDTA, 100 mM NaCl, 1 mM DTT, 0.1 mM PMSF, 3.3 μg/mL leupeptin, 3.2 μg/mL soybean trypsin inhibitor, 2 μg/mL aprotinin). Cells were lysed by lysozyme (0.2 mg/mL). Cleared lysates were incubated for 1 h at 10 °C with 26 μL of glutathione−Sepharose 4B (Pharmacia) in buffer A including 10 mM $MgCl_2$ and 0.01% $C_{12}E_{10}$ (final volume 1 mL). Sepharose beads were washed with buffer B (20 mM Na-Hepes, pH 8, 2 mM EDTA, 10 mM $MgCl_2$, 100 mM NaCl, 0.01% $C_{12}E_{10}$, 1 mM DTT) and incubated with 180 nM $G\beta\gamma$ in buffer B for 1 h at 10 °C (final volume 250 μL). Proteins were eluted from the beads by incubation for 20−30 min at 25 °C in 50 mM Tris-HCl, pH 8.8/15 mM glutathione/0.01% $C_{12}E_{10}$. Eluted proteins were separated on a 12% SDS−PAG and analyzed for fusion proteins by Coomassie blue stain and for $G\beta\gamma$ binding by immunostain with a $G\beta$-specific antiserum (*18*).

Miscellaneous. AC activity was determined using [α-^{32}P]-ATP as substrate (*19*). Reactions contained 10 mM $MgCl_2$, 1.2 mM CHAPS, and 10 μg/mL BSA and were performed with 5 μg of protein in membrane or soluble fractions. For peptide competition experiments peptides were mixed with $G\beta\gamma$ in Tris-HCl, pH 7.5, 1 mM EDTA, 1.2 mM CHAPS, 10 μg/mL BSA, and 1 mM DTT and incubated for 1 h on ice before $G\alpha_s$ and membranes were added. Immunodetection of tagged AC fragments was performed with monoclonal antibodies (anti-HA:12CA5, Roche; anti-GST:B-14, Santa Cruz; anti-c-MYC:9E10, Santa Cruz; anti-RGSHis, RGSHis antibody, Qiagen).

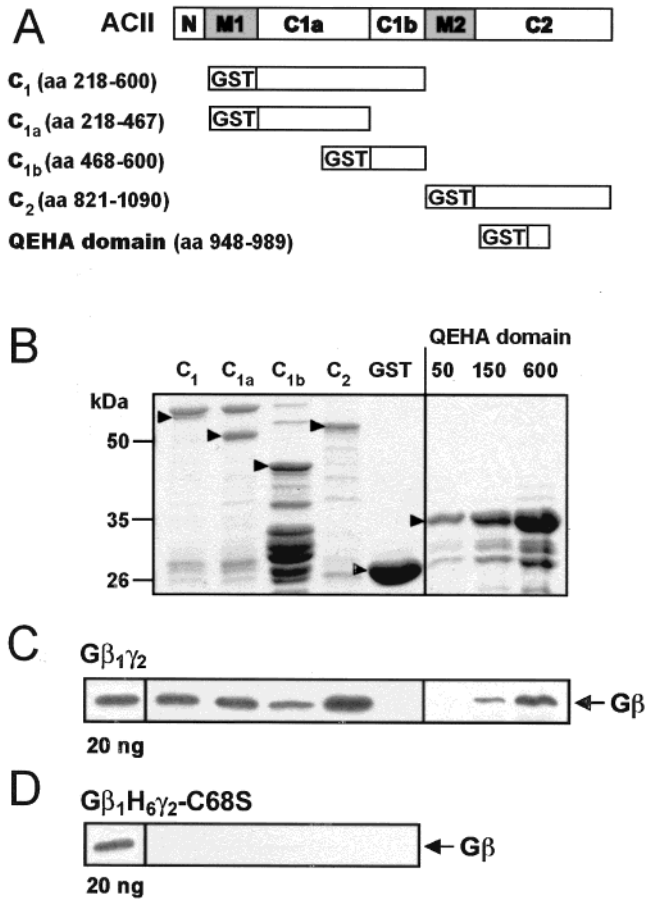

FIGURE 1: $G\beta\gamma$ binding to cytosolic domains of ACII. GST fusion proteins of cytosolic domains of ACII were designed as illustrated in (A) and expressed in *E. coli*. $G\beta\gamma$ binding was determined for each construct derived from the soluble fraction as described under Materials and Methods. (B) Coomassie blue stain of lysates enriched on glutathione−Sepharose. Fusion proteins were detected by immunostaining with GST-specific antibodies (not shown), and the corresponding protein bands are marked with an arrow. The QEHA domain was supplied as 50, 150, or 600 μL of the soluble fraction from recombinant bacteria expressing this fusion protein. (C, D) Immunostain for $G\beta$. As a control, 20 ng of each of the $G\beta\gamma$ complexes used was applied to the same PAG (left lane). Experiments were performed with $G\beta_1\gamma_2$ (C) or $G\beta_1H_6\gamma_2$-C68S (D). Data are representative of two to four independent experiments.

RESULTS AND DISCUSSION

Gβγ Interaction with Cytosolic Domains of ACII. To date, only one region of ACII is known to interact with $G\beta\gamma$ (*11*, *12*). This region comprises 27 amino acids of the C_2 domain (aa 956−982) and will be referred to in the following as the QEHA region (Figure 3A). On the basis of the crystal structure of AC's catalytic core, mechanisms for $G\alpha_s$ and forskolin regulation have been deduced (*10*, *20*). A model for regulation by $G\beta\gamma$ has not been established. Besides the

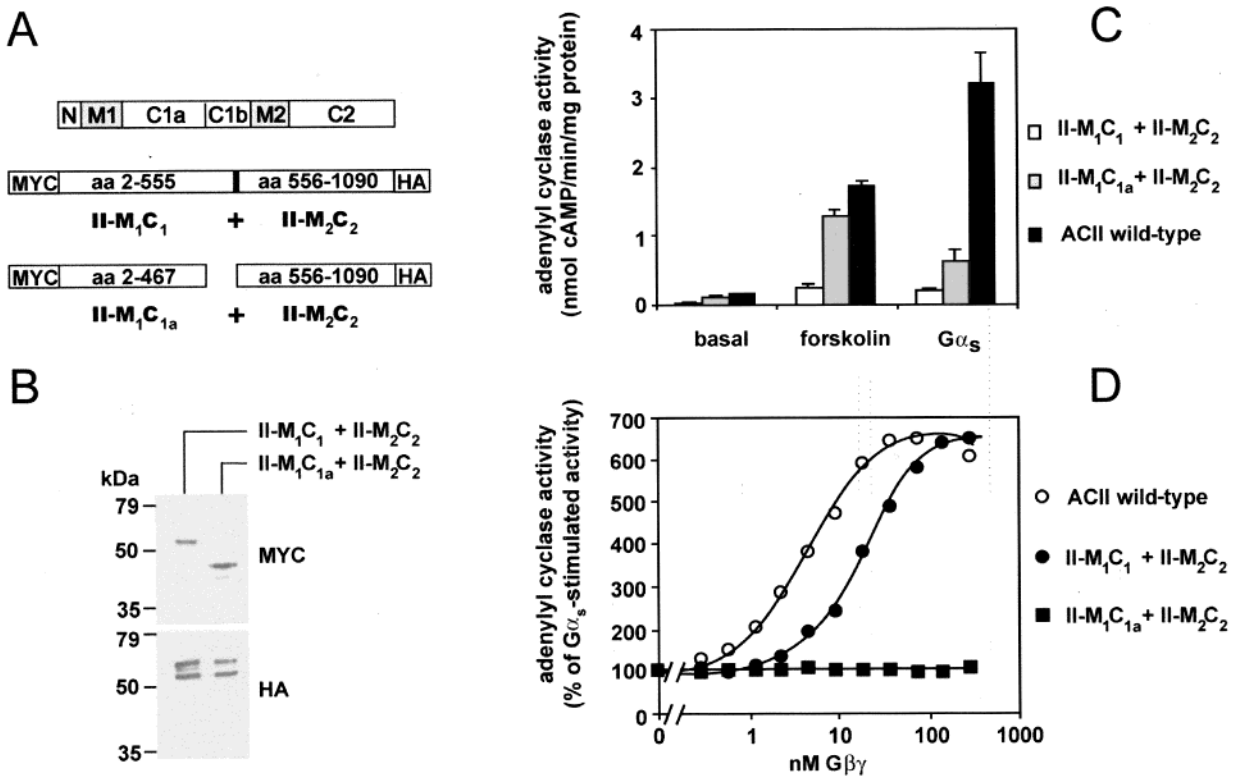

FIGURE 2: Influence of the C_{1b} domain on G$\beta\gamma$ stimulation of ACII. ACII mutants were constructed as illustrated in (A) and expressed in Sf9 cells. (B) Two micrograms of protein of membranes from Sf9 cells coexpressing II$-M_2C_2$ with either II$-M_1C_1$ or II$-M_1C_{1a}$ was separated on a 10% SDS−PAG. Proteins were transferred to nitrocellulose membranes and immunostained with MYC- or HA-selective antibodies as indicated. Double bands on the HA-selective filter represent the glycosylated and nonmodified forms of II$-M_2C_2$ (*13*). (C) Basic characterization of AC constructs. Membranes from Sf9 cells expressing bisected AC (II$-M_1C_1$ + II$-M_2C_2$), C_{1b}-truncated bisected ACII (II$-M_1C_{1a}$ + II$-M_2C_2$), or wild-type ACII were incubated without or with 80 nM Gα_s or 100 μM forskolin. Values are means ± SEM of three independent experiments performed in duplicate. Data are corrected for endogenous AC activity [Sf9 cells expressing β-galactosidase, 0.168 nmol of cAMP min^{-1} (mg of protein)$^{-1}$]. (D) G$\beta\gamma$ regulation of AC constructs. Membranes from Sf9 cells expressing wild-type ACII, coexpressing II$-M_1C_1$ with II$-M_2C_2$ or II$-M_1C_{1a}$ with II$-M_2C_2$, were incubated with the indicated amounts of G$\beta\gamma$ in the presence of 80 nM Gα_s. Data are representative of four similar experiments performed in duplicate.

QEHA region, other interaction sites of G$\beta\gamma$ were supposed to exist in ACII. Therefore, we investigated G$\beta\gamma$ binding to cytosolic domains using GST fusion proteins with C_1, C_{1a}, C_{1b}, or C_2 of ACII. A fusion protein comprising a 42 amino acid fragment of II$-C_2$ encompassing the QEHA region in the center was also generated (QEHA domain, Figure 1). All fusion proteins specifically interacted with G$\beta\gamma$. This was evident for wild-type G$\beta_1\gamma_2$ and also a complex with N-terminally tagged Gγ_2 (G$\beta_1H_6\gamma_2$, not shown). G$\beta\gamma$ binding to the QEHA domain required more fusion protein than binding to any other cytosolic domain. We are aware that this experimental approach is not appropiate to determine affinities in exact numbers: The GST−AC segments may carry attached *E. coli* chaperones or heat shock proteins to variable extents, thereby changing G$\beta\gamma$ affinities for individual fused segments. We consider this unlikely because (i) we performed an enzymatic lysis of the bacteria without sonication in order to avoid such denaturation-provoked effects and (ii) the staining pattern of lysates containing the GST-fused QEHA domain did not reveal stoichiometric amounts of any copurified protein of appropriate molecular mass (>35 kDa). The major contaminant of approximately 70 kDa comprised less than 5% of total protein in the lysate and is probably the *E. coli* chaperonin DnaK.

No binding of G$\beta\gamma$ to any immobilized AC was observed when a nonprenylated mutant form of Gγ_2 was used (H_6Gγ_2-C68S, ref *21*). These data provide strong evidence for the presence of at least three G$\beta\gamma$-binding sites on ACII, located on C_{1a}, C_{1b}, and C_2. Moreover, direct interaction of G$\beta\gamma$ with cytosolic AC domains seems to be restricted to complexes containing the lipid-modified Gγ_2 subunit. A lipid-dependent activation, i.e., functional interaction, by G$\beta\gamma$ has already been described for wild-type ACII (*22*). Obviously, isoprenylation of Gγ_2 has a regulatory role that goes beyond mere anchoring of the G$\beta\gamma$ complex to the plasma membrane close to the AC effector.

Involvement of the C_{1b} Domain. To elucidate the functional consequence of the novel interaction of G$\beta\gamma$ with C_1, we coexpressed in Sf9 cells N- and C-terminal halves of ACII containing the entire C_1 domain or the C_{1a} subdomain (Figure 2). As described previously (*13*), the extent of forskolin and Gα_s stimulation on bisected ACII (II$-M_1C_1$ + II$-M_2C_2$) was greatly diminished compared to the wild-type enzyme. Nevertheless, G$\beta\gamma$ stimulated the bisected ACII to the same extent as wild-type ACII although affinity was reduced about 10-fold. Coexpression of II$-M_1C_{1a}$ with II$-M_2C_2$ resulted in a particulate, bisected ACII that lacked the N-terminal 89 amino acids of II$-C_{1b}$. Deletion of C_{1b} in the bisected ACII restored the stimulatory efficacy of forskolin and, to a lesser extent, that of Gα_s. In contrast to these rescuing effects on forskolin and Gα_s stimulation, deletion of C_{1b} resulted in almost complete loss of G$\beta\gamma$ regulation in bisected ACII (see Figure 2D). These data revealed that the C_{1b} domain plays an important role in mediating the stimulatory response

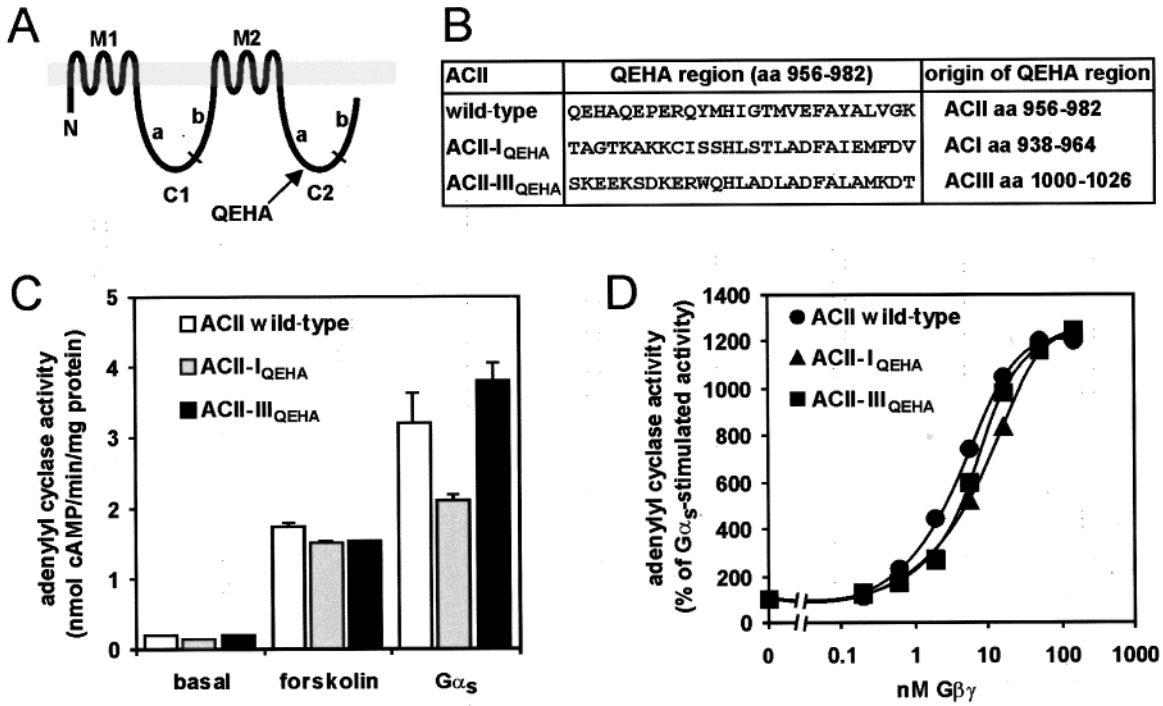

FIGURE 3: Effect of QEHA mutations on regulation of ACII. (A) Predicted topology of membrane-bound mammalian ACs. Abbreviations: N, cytosolic N-terminus; M_1 and M_2, first and second set of six transmembrane spans; C_1 and C_2, first and second cytosolic domain with conserved C_{1a}, C_{2a} and variable C_{1b}, C_{2b} subdomains. An arrow indicates the site of the QEHA region. (B) Schematic description of generated substitutions in ACII. The QEHA region in ACII (aa 956−982) was replaced by the analogous regions of ACI (aa 938−964) and ACIII (aa 1000−1026) to generate ACII mutants ACII−I_{QEHA} and ACII−III_{QEHA}, respectively. (C) Basic characterization of AC constructs. Membranes from Sf9 cells expressing wild-type ACII, ACII−I_{QEHA}, or ACII−III_{QEHA} were incubated without or with 80 nM $G\alpha_s$ or 100 μM forskolin. Values are means ± SEM of three independent experiments performed in duplicate. Data were corrected for endogenous AC activity [Sf9 cells expressing β-galactosidase, 0.153 nmol of cAMP min^{-1} (mg of protein)$^{-1}$]. (D) $G\beta\gamma$ regulation of AC constructs. Membranes from Sf9 cells expressing wild-type ACII, ACII−I_{QEHA}, or ACII−III_{QEHA} were incubated with the indicated amounts of $G\beta\gamma$ in the presence of 20 nM $G\alpha_s$. $G\alpha_s$-stimulated activities (100%) were 1.9 nmol of cAMP min^{-1} (mg of protein)$^{-1}$ (ACII wild-type), 1.2 nmol of cAMP min^{-1} (mg of protein)$^{-1}$ (ACII−I_{QEHA}), and 2.6 nmol of cAMP min^{-1} (mg of protein)$^{-1}$ (ACII−III_{QEHA}). Shown are the data from one of three similar experiments performed in duplicate.

of ACII upon $G\beta\gamma$ binding. The important impact of the C_{1b} region on AC activity also became evident when comparing enzyme activities of various soluble, membrane-anchored and bisected AC constructs: The mere presence of the C_{1b} region reduced AC activity (basal, $G\alpha_s$- or forskolin-stimulated activity, ref *23*; unpublished results for ACII constructs). Therefore, any interaction of a protein with this intrinsic regulator of AC activity, e.g., calmodulin with ACI or $G\beta\gamma$ with ACII (*24*, *25*), is anticipated to alter this inhibition constraint on the enzyme and the catalytic process.

In subsequent experiments we have investigated the relevance of $G\beta\gamma$ interaction (physical contact) with the QEHA region for the stimulatory effect (functional contact) that $G\beta\gamma$ exerts on ACII.

Involvement of the QEHA Region in Gβγ Regulation of ACII. It is well-known that the QEHA region of ACII is an interaction site for $G\beta\gamma$ (*12*), but it has not been determined yet whether the QEHA region is also responsible for the stimulatory response of ACII upon $G\beta\gamma$ binding. We have substituted the QEHA region of ACII by the analogous regions of ACI (inhibited by $G\beta\gamma$) or ACIII (unresponsive to $G\beta\gamma$) to generate the mutants ACII−I_{QEHA} or ACII−III_{QEHA}, respectively (Figure 3). Wild-type ACII and both mutant ACs were expressed in Sf9 cells and were similarly active in the basal and forskolin-stimulated state but exhibited modest differences in their regulation by $G\alpha_s$. Surprisingly, $G\beta\gamma$ stimulated both mutants. We did not observe significant differences of $G\beta\gamma$, neither in potency nor efficacy, to stimulate wild-type ACII or either mutant.

Like ACII, ACI is also a $G\beta\gamma$-regulated effector; hence, we could not exclude that ACII−I_{QEHA} contained the $G\beta\gamma$ interaction site of ACI, which substituted for the docking feature of the QEHA region in ACII. Likewise, it is possible that ACIII physically interacts with but is not regulated by $G\beta\gamma$. To test the hypothesis of a $G\beta\gamma$ interaction site on ACI and ACIII, we generated peptides covering the substituted sequences illustrated in Figure 3B. Peptides were tested for their abilities to suppress the $G\beta\gamma$ regulation of ACI and ACII. The TAGT peptide represents the region of ACI that corresponds to that in ACII covered by the QEHA peptide. The TAGT peptide reduced the $G\beta\gamma$-dependent regulation of ACI (circular symbols in Figure 4A) and ACII (Figure 4B) as efficiently as the QEHA peptide (triangular symbols in Figure 4). This finding was surprising because the TAGT peptide did not contain a QXXER motif that had been described to be crucial for interaction of the QEHA peptide with $G\beta\gamma$. In contrast, the SKEE peptide representing the QEHA-corresponding region of the $G\beta\gamma$-unresponsive ACIII did not affect $G\beta\gamma$ regulation of ACI or ACII. This confirmed recent data of Chen et al. (*11*), who concluded that the QEHA region in ACII represents a binding site for $G\beta\gamma$. Furthermore, we conclude that the QEHA analogous region, represented by the TAGT peptide, serves as a $G\beta\gamma$ binding domain in ACI.

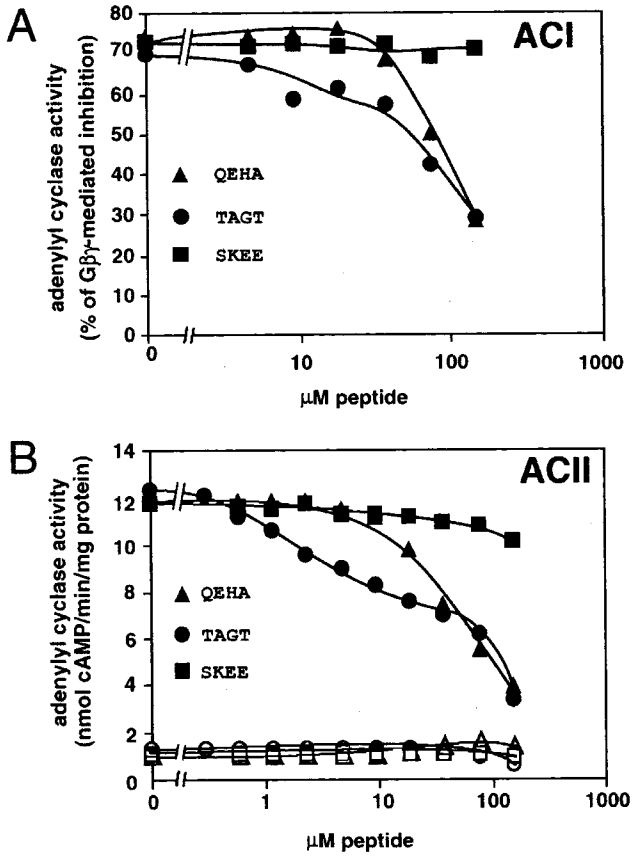

FIGURE 4: Effect of QEHA and QEHA analogous peptides on Gβγ regulation of AC. Peptides composed of amino acids denoted in Figure 3B were used for competition experiments of Gβγ-regulated ACs. (A) Gβγ-mediated inhibition of ACI. Increasing amounts of QEHA (ACII derived), TAGT (ACI derived), or SKEE (ACIII derived) peptides were incubated with membranes from Sf9 cells expressing ACI in the presence of 100 nM $G\alpha_s$ without or with 50 nM Gβγ. Values are expressed as the difference of $G\alpha_s$-stimulated (*S*) and $G\alpha_s$- plus Gβγ-regulated AC activity (*SB*) related to $G\alpha_s$-stimulated AC activity: $(S - SB)/S$. Mean values of AC activity without peptide were 2.28 nmol of cAMP min^{-1} (mg of protein)$^{-1}$ ($G\alpha_s$ stimulation) and 0.64 nmol of cAMP min^{-1} (mg of protein)$^{-1}$ ($G\alpha_s$ plus Gβγ). (B) Gβγ-mediated activation of ACII. Peptides were incubated with membranes from Sf9 cells expressing ACII in the presence of 20 nM $G\alpha_s$ (open symbols) or 20 nM $G\alpha_s$ plus 50 nM Gβγ (solid symbols). All data are representative of at least three similar experiments performed in duplicate.

It was striking that $ACII-III_{QEHA}$ displayed the regulatory properties of the wild-type enzyme, although the III_{QEHA} corresponding SKEE peptide did not block Gβγ-mediated regulation of AC. One explanation may be a folding problem of the SKEE peptide. Taken together, we would like to draw the conclusion that the QEHA region per se displayed only a modest affinity for Gβγ, as indicated by the direct Gβγ-binding assays (see Figure 1C) and by the high peptide concentrations necessary for competition experiments (see Figure 4). Although interacting with Gβγ in vitro, the QEHA region may be of minor importance for Gβγ binding to the holoenzyme. Similar conclusions can be drawn from the random peptide phage display screening performed by Scott et al. (*26*). Using 16 libraries, they cleverly sorted out 19 different peptides that interacted with functional Gβγ: none of these peptides comprised the QEHA region. This screening approach may be limited to high-affinity interaction sites and revealed four classes of hydrophobic peptides that obviously bind to the same site on Gβγ. We have shown that Gβγ interacts at several sites with AC, what probably is reflected by several binding sites on Gβγ, including one for interaction with the QEHA domain. Taking into account that many Gβγ effectors, including PIP_3 kinase γ, phospholipase $C\beta_2$, and ACI, do not contain the QEHA consensus motif (QXXER), the QEHA domain may not represent a universal motif. In terms of the functional aspect of this interaction, our data do not support a role of QEHA in stimulating ACII upon Gβγ binding. Recently, Buck and co-workers resolved in Gβ a signal transfer region from a general binding domain (*27*). ACII could also provide distinct regions responsible for signaling and binding. If this model is applicable, the QEHA region may represent a general docking site for Gβγ but not a major signal transfer region for ACII stimulation. Even in the context with the C_2 domain plus C_{1a} of ACII (see C_{1b} deletion mutant of ACII), QEHA was unable to transmit the full stimulatory signal upon Gβγ binding.

Obviously, the C_{1a} regions are differentially involved in the Gβγ-mediated regulation of ACs. Wittpoth and co-workers described the C_{1a} domain of ACI to be sufficient for mediating Gβγ inhibition of a soluble AC chimera (ACI−V; ref *28*). In contrast, C_{1a} was not sufficient to allow a Gβγ-mediated stimulation in the C_{1b}-deleted mutant of ACII. So far, ACs provide multiple contact sites for Gβγ, i.e., C_{1a}, C_{1b} and C_2, but depending on the isoform, each contact site may differ in its relative importance as a docking site or as a site that transmits the regulatory signal.

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