

Mapping regions of $G_{\alpha q}$ interacting with $PLC\beta 1$ using multiple overlapping synthetic peptides

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Abstract The heterotrimeric G-protein α -chain $G_{\alpha q}$ plays a critical role mediating receptor-linked activation of the β isoforms of PLC which hydrolyse membrane inositol-containing phospholipids to generate the second messengers inositol 1,4,5-trisphosphate and diacylglycerol. Despite knowledge of the three-dimensional structure of two G-protein α -chains ($G_{\alpha i}$ and $G_{\alpha 11}$) as well as high regional amino acid conservation between members of the G-protein α -chain family, the precise molecular domains of $G_{\alpha q}$ mediating activation of $PLC\beta 1$ are unknown. To map sites responsible for effector interaction we employed 188 peptides each of 15 residues and corresponding to overlapping regions of the complete $G_{\alpha q}$ sequence. These were tested for their ability to inhibit $G_{\alpha q}$ -dependent activation of recombinant $PLC\beta 1$ using an in vitro reconstitution assay. Peptides from two regions of $G_{\alpha q}$ mediated up to 100% inhibition of GTP γ S-stimulated $PLC\beta 1$ activity, and representative peptides from each of these regions were half-maximally effective at $69.3 \pm 27.4 \mu M$ ($n = 4$) ($G_{\alpha q}$: 251–265) and $110.0 \pm 41.9 \mu M$ ($n = 4$) ($G_{\alpha q}$: 306–319). $G_{\alpha q}$ regions described by inhibitory peptides are conserved selectively in other G-protein α -chains linked to $PLC\beta 1$ activation ($G_{\alpha 11}$, $G_{\alpha 14}$) and correspond spatially to sites of effector interaction identified in $G_{\alpha s}$ by scanning mutagenesis and in transducin using site-specific antibodies and peptides. Computer homology modelling of $G_{\alpha q}$ based on the crystal structure of transducin indicates that regions interacting with $PLC\beta 1$ form two parallel α -helices lying at the surface of the $G_{\alpha q}$ structure. These observations provide the first description of two regions within $G_{\alpha q}$ critically important for activating $PLC\beta 1$, and moreover, indicate that effector binding domains identified in transducin and $G_{\alpha s}$ are also conserved spatially in $G_{\alpha q}$.

Key words: G-protein; $G_{\alpha q}$; $PLC\beta$; Phosphoinositide hydrolysis; Synthetic peptide; Protein modelling; Effector interaction

1. Introduction

Receptors of the seven transmembrane superfamily play a critical role detecting diverse extracellular signals [1,2]. They control a multitude of cell functions through activating heterotrimeric G-proteins which in turn trigger regulation of a selected repertoire of downstream effector and second messenger systems [1,2]. Recent determination of the three-dimensional structure of two G-protein α -chains ($G_{\alpha i}$ and $G_{\alpha 11}$) reveals con-

siderable molecular detail of activation-dependent conformational changes as well as core residues likely to play crucial roles in GTP binding and hydrolysis [3–6]. These crystal structures also identify domains forming contiguous sequences at the molecular surface and therefore of potential importance for regulatory interactions with receptor, G-protein $\beta\gamma$ dimers and effector targets. To identify unequivocally sites critical for G-protein α -subunit interaction with other regulatory components it is necessary to combine analysis of G-protein structure with studies designed to probe the functional importance of specific molecular domains. This has been illustrated by investigations with pertussis toxin [7], site-directed antibodies [8–11], synthetic peptides [12,13], as well as analysis of mutations [14–17] which have identified the carboxyl-terminal of many α -chains as an important site for receptor interaction. Together with analysis of three-dimensional structure, this information provides invaluable insight into both protein–membrane orientation and molecular contacts at the receptor/G-protein interface.

An additional protein–protein interaction of fundamental importance within G-protein linked signal transduction pathways is that between α -chains and downstream effector targets. One second messenger system of critical importance for cell regulation is increased breakdown of inositol-containing phospholipids by phospholipase C (PLC). This generates the second messengers inositol 1,4,5-trisphosphate and diacylglycerol which mobilize Ca^{2+} stores and activate protein kinase C, respectively [18,19]. Molecular dissection of the major components underlying this pathway implicate G-protein α -chains of the G_q class and moreover, have identified β isoforms of PLC as specific effector targets for regulatory activation [2,20–25]. Despite the clear recognition of G_q α -chains as important regulators of phosphoinositide hydrolysis, we remain ignorant of the precise structural domains and sites of interaction underlying activation of $PLC\beta$. For other G-protein systems mutational analysis and peptides have been used to probe regions within $G_{\alpha s}$ and $G_{\alpha i}$ important in mediating activation of their respective effector targets, adenylyl cyclase and cyclicGMP phosphodiesterase [26–29]. However, no such studies have reported the identity of key residues within the G_q class of α -chains in their interaction with $PLC\beta$. We report here the use of synthetic peptides corresponding to overlapping regions of the entire sequence of $G_{\alpha q}$ which were tested for their ability to block activation of recombinant $PLC\beta 1$ by GTP γ S-ligated $G_{\alpha q}$. This approach identified clusters of peptides from two distinct regions of $G_{\alpha q}$ which mediated inhibition of $G_{\alpha q}$ -linked $PLC\beta 1$ activation. Although separated in linear sequence, computer modelling reveals that these peptides describe two α -helical domains lying parallel to one another at the surface of $G_{\alpha q}$.

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2. Experimental

2.1. Peptide synthesis and purification

Peptides of 15 amino acids corresponding to overlapping regions (with steps of one or two residues) from the entire mouse G_{aq} sequence [30] were custom synthesized by Chiron Mimotopes (Clayton, Victoria, Australia) using multiple pin synthesis technology. All peptides were acetylated and amidated at the amino- and carboxy-termini, respectively. In total, 188 individual peptides were tested for inhibition of GTP γ S-stimulated PLC β 1 activation (see below) at a final concentration of $\sim 500 \mu\text{M}$. For further analysis of inhibitory activity, selected peptides (as indicated) were resynthesized by Neosystem Laboratoire (Strasbourg, France). These were purified to $>95\%$ by reverse phase high pressure liquid chromatography using a Nucleosil 300-7 C8 column (Machery-Nagel ET 250/8/4); buffer A, 0.1% (w/v) TFA/water; buffer B, 0.09% TFA/80% acetonitrile/water; flow rate, 1.0 ml/min and a 0–75% gradient of buffer B over 60 min.

2.2. Strains, media and plasmid constructs

The host strain for *S. pombe* transformation with PLC β 1, h^+ , $leu1-32$, $ura4-d18$, $ade6-704$, $his5-303$ was based on the original wild type 975h $^+$ strain from the Berne collection and constructed using standard genetic procedures [31]. Growth media and the procedure for protoplast transformation are as described [32]. The bacterial strain for routine work was JM101TR (*supE*, *thi* $^-$, *src::tn10*, *recA* Δ (lac-proAB), [F' , traD36, proAB, lacZ Δ M15]) and standard media were used throughout. The G_{aq} and PLC β 1 cDNAs were provided by Professor M.I. Simon (California Institute of Technology, Pasadena) and Dr. P. Parker (Imperial Cancer Research Fund, London), respectively, and were as reported [30,33]. For expression in fission yeast, coding sequences from the PLC β 1 cDNA was excised using appropriate restriction enzymes and subcloned into the *mtl1*-based expression vector pREP3 [34]. G_{aq} expression in baculovirus-infected *Spodoptera frugiperda* (Sf9) cells was performed as described [35].

2.3. Preparation of yeast homogenates

Suitably transformed *S. pombe* cells were grown overnight to stationary phase in minimal medium containing 4 μM thiamine after which they were diluted to 5×10^5 cells/ml in fresh minimal medium and cultured with or without exogenously added thiamine for another 18 h until late log-phase ($1-2 \times 10^7$ /ml). For homogenization yeast cells were washed in 0.9% (w/v) NaCl and broken by vigorous vortexing for 4 \times 1 min at 4°C in the presence of 1.5 ml of glass beads (500 μm diameter) and 100 μl of buffer P (10 mM Tris-HCl, 1 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 10 $\mu\text{g}/\text{ml}$ leupeptin, 10 $\mu\text{g}/\text{ml}$ chymostatin, 10 $\mu\text{g}/\text{ml}$ aprotinin, pH 7.4). A further 400 μl of buffer P was then added and the homogenate recovered by low speed centrifugation after piercing the base of the tube. The homogenate was further centrifuged at $1000 \times g$ for 5 min to pellet unbroken cells and debris. The supernatant containing both particulate material and soluble proteins was designated crude homogenate. Using this protocol 1×10^8 cells yields routinely crude homogenates with a protein content of 10–15 mg/ml.

2.4. Measuring PLC β 1 activity with exogenous substrate

For G_q preactivation, crude homogenates of G_{aq} -expressing baculovirus infected Sf9 cells or transformed *S. pombe* were diluted 5- to 10-fold in buffer C (5 mM Tris-HCl, 10 mM MgCl $_2$, 1 mM dithiothreitol (DTT), pH 7.2) containing GTP γ S or GDP β S and incubated at 37°C for 60 min. This was used as a source of G_q for subsequent incubation with PLC β 1 (see below). To prepare exogenous substrate to measure PLC β 1 activity, approximately 1 μCi of [*inositol*-2- ^3H]phosphatidylinositol 4,5-bisphosphate (^3H]PIP $_2$; 8.0 Ci/mmol) (New England Nuclear; Regensdorf, Switzerland) was mixed with 110 μl each of phosphatidylethanolamine and phosphatidylserine from bovine brain (10 mg/ml; Sigma Chemie, Buchs, Switzerland) in glass tubes and dried under vacuum with centrifugation. Following addition of 45 μl of 5 mM PIP $_2$ from bovine brain (Fluka Chemie AG, Buchs, Switzerland) dissolved in 50 mM HEPES, 100 mM KCl and 10 mM deoxycholate (pH 7.0), phospholipids were resuspended in 1.0 ml of 20 mM HEPES, pH 7.2, containing 100 mM NaCl using a bath sonicator for 5 min followed by 4 \times 10 s with a probe sonicator at full power. Aliquots of substrate (25 μl) were added to glass tubes together with 25 μl of buffer B (50 mM Tris-HCl, 50 mM maleic acid, 40 mM LiCl and 20 mM MgCl $_2$ at

pH 7.0) containing CaCl $_2$ at concentrations calculated to give defined free Ca $^{2+}$ levels between 10^{-8} M and 10^{-3} M (based on 1 mM ethylene glycol-bis (β -aminoethyl ether)-N,N,N',N'-tetra acetic acid (EGTA)). To initiate reactions, 25 μl of preactivated G_{aq} in Sf9 cell homogenates followed by 25 μl of PLC β 1 extract (from *S. pombe* transformed with PLC β 1 and diluted 15-fold in 5 mM Tris-HCl, 4 mM EGTA, 1 mM DTT, pH 7.2) were added, vortex mixed, and incubated at 35°C for 30 min in a shaking water bath. When G_{aq} peptides were tested these were added as 5 μl aliquots dissolved in DMSO. Reactions were stopped by adding 1.2 ml of an ice-cold mixture of methanol:chloroform:HCl (200:100:1 (v/v)) followed by a further 0.5 ml of chloroform, 0.5 ml of 0.25 M HCl and vortex mixing. Samples were then centrifuged at $1000 \times g$ for 5 min and 0.5 ml of upper aqueous phase counted for radioactivity by scintillation spectrometry.

2.5. Molecular modelling of G_{aq}

The murine G_{aq} sequence was from SwissProt (entry GBQ_MOUSE; Accession number: P21279) while the bovine G_{at} structural co-ordinates were obtained through the Brookhaven Protein Data bank (entry 1TND). The sequences of mouse G_{aq} and G_{at} were aligned with SIM [36]. The modelling procedure for G_{aq} involved steps implemented in the automated protein modelling package ProMod [37]: (i) the generation of a framework for all atoms; (ii) the rebuilding of two loops with a one residue deletion; (iii) rebuilding of missing backbone atoms; and (iv) the addition of missing side chains and their optimisation. Subsequent optimisation of bond geometry and relief of unfavourable non-bonded contacts was performed by 30 steps of steepest descent followed by 500 steps of conjugate gradient minimisation using the CHARMM package [38] with the PARAM19 parameter set. After visual inspection some surface side chains were moved to more suitable rotamers and the dimer structure was further optimised by 2000 steps of conjugate gradi-

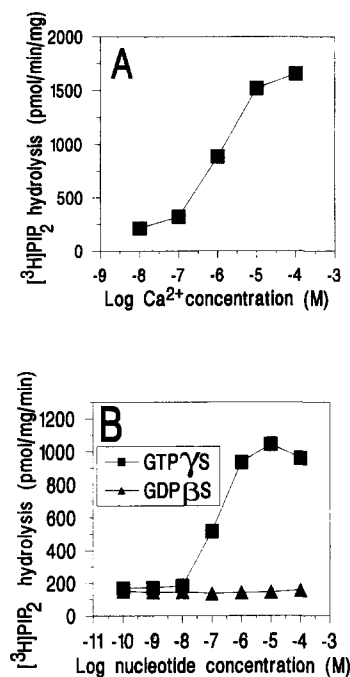


Fig. 1. Recombinant PLC β 1 produced in fission yeast is activated by Ca $^{2+}$ and by G_{aq} produced in baculovirus-infected Sf9 insect cells. *S. pombe* transformed with pREP3/PLC β 1 was grown for 18 h in the absence of thiamine after which time homogenates were prepared by cell breakage with glass beads. (A) Hydrolysis of exogenous [^3H]PIP $_2$ to generate [^3H]IP $_3$ by yeast extracts expressing PLC β 1 in the presence of increasing concentrations of free Ca $^{2+}$. Control cells gave no detectable [^3H]IP $_3$ hydrolysis under identical conditions. (B) PLC β 1 stimulated by G_{aq} produced by baculovirus-infected Sf9 cells preactivated by incubation in the presence of increasing concentrations of GTP γ S. Free Ca $^{2+}$ levels were maintained at 10^{-7} M. GDP β S pretreated Sf9 extracts are unable to stimulate PLC β 1 activation. Data points are the mean of triplicate determinations and are representative of at least 3 independent experiments.

ent minimisation. The 3D–1D profile matching procedure of Lüthy et al. [39] was used to assess the quality of the models.

3. Results and discussion

Bovine PLC β 1 subcloned into pREP1 for expression in fission yeast (see section 2) was immunodetectable as a 150 kDa protein localized predominantly in membrane fractions (not shown). Yeast extracts hydrolysed exogenously supplied [3 H]PIP $_2$ and this activity was stimulated markedly by increasing levels of free Ca $^{2+}$ in the concentration range 10^{-7} to 10^{-5} M (Fig. 1A). 100% of the [3 H]PIP $_2$ hydrolytic product co-eluted with authentic [3 H]IP $_3$ following separation using anion-exchange resin mini-columns (data not shown). No PLC activity was detectable under identical conditions using control cells expressing pREP vector alone (data not shown). As reported recently [40], G $_{aq}$ expressed in baculovirus-infected Sf9 insect cells stimulates PLC β 1 activation following preactivation by incubation in the presence of increasing concentrations of GTP γ S (Fig. 1B). GDP β S was ineffective under identical conditions.

To map molecular domains within G $_{aq}$ responsible for interaction with PLC β 1 we measured GTP γ S-stimulated PLC β 1 activation in the presence of synthetic peptides corresponding

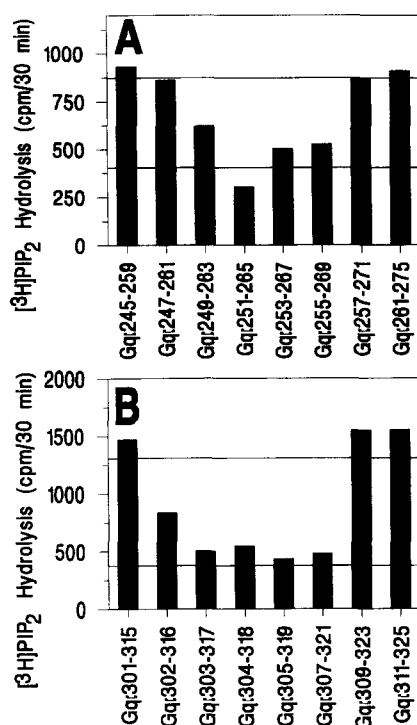


Fig. 2. Inhibition of G $_{aq}$ -stimulated PLC β 1 activity by synthetic peptides corresponding to 15 amino acid sequences within G $_{aq}$. PLC β 1 was activated by Sf9 cell extracts expressing G $_{aq}$ preincubated in the presence of 10^{-4} M GTP γ S and [3 H]PIP $_2$ hydrolysis measured in the presence of overlapping synthetic peptides from G $_{aq}$ as indicated. A total of 188 peptides were tested independently at a final concentration of 500 μ M. Two clusters of inhibitory activity were detected for peptides corresponding to amino acids 249–269 (A) and 302–321 (B) of the G $_{aq}$ primary amino acid sequence. Lower and upper horizontal lines indicate unstimulated and G $_{aq}$ -dependent PLC β 1 activity measured in the absence of peptide. Bars represent the mean of duplicate determinations for peptides inhibiting PLC β 1 activation in at least two separate experiments.

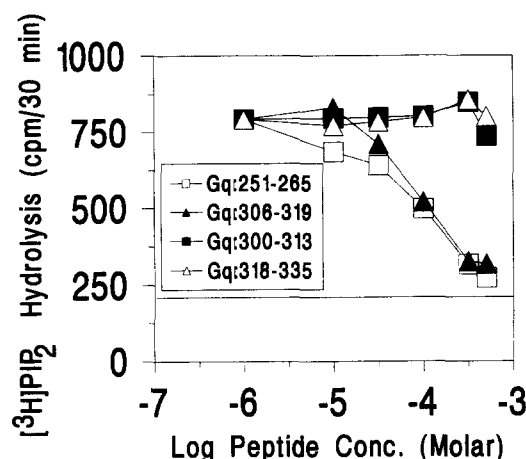


Fig. 3. Dose-dependent inhibition of G $_{aq}$ -dependent PLC β 1 activity by purified synthetic peptides: G $_{aq}$: 251–265 (SKALFRTIITYPWQ) and G $_{aq}$: 306–319 (AREFILKMFVDLNP). [3 H]PIP $_2$ hydrolysis by G $_{aq}$ -activated PLC β 1 was measured in the presence of increasing concentrations of HPLC-purified synthetic G $_{aq}$ peptides as indicated. G $_{aq}$: 251–265 and G $_{aq}$: 306–319 mediated complete inhibition of GTP γ S-activated PLC β 1 while G $_{aq}$: 300–313 and G $_{aq}$: 318–335 were ineffective at concentrations up to 500 μ M. Horizontal line indicates basal PLC β 1 activity measured in the absence of GTP γ S-stimulated G $_{aq}$. Points are the mean of duplicate determinations and representative of 4 separate determinations. Identical results were obtained using two separate batches of peptide and independent HPLC purifications.

to overlapping sequences for the entire G $_{aq}$ molecule [30]. We tested 188 peptides, each of 15 amino acids at a final concentration of 500 μ M. We observed two major clusters of peptides inhibiting G $_{aq}$ -dependent PLC β 1 activation by up to 100%. Peptides clearly inhibitory on G $_{aq}$ function correspond to residues 249–269 and 302–321 of the G $_{aq}$ sequence (Fig. 2A,B). To confirm these inhibitory properties two representative peptides, G $_{aq}$: 251–265 (SKALFRTIITYPWQ) and G $_{aq}$: 306–319 (AREFILKMFVDLNP), were resynthesized and purified using HPLC. Both G $_{aq}$: 251–265 and G $_{aq}$: 306–319 inhibited G $_{aq}$ -stimulated PLC β 1 activity in a dose-dependent fashion displaying IC $_{50}$ values of 69.3 ± 27.4 μ M ($n = 4$) and 110.0 ± 41.9 μ M ($n = 4$), respectively (Fig. 3). These concentrations are similar to those required for peptides from the carboxyl-terminal of G $_{as}$ and G $_{at}$ for blockade of G-protein interaction with receptor [12,13,41]. Two purified control peptides corresponding to regions G $_{aq}$: 300–313 (RDAQAAREFILKKMF) and G $_{aq}$: 318–335 (PDSKIIYSSHFTCATDTE) which overlap with the amino- and carboxyl-termini of G $_{aq}$: 306–319 were both ineffective at blocking G $_{aq}$ action at any concentration up to 500 μ M (Fig. 3).

It may be expected that the inhibitory peptides G $_{aq}$: 251–265 and G $_{aq}$: 306–319 exhibit the ability to fold into a three-dimensional conformation mimicking cognate regions on the surface of the G $_{aq}$ thereby competing for interaction with recognition sites on PLC β 1. In order to assess the molecular localization of these inhibitory peptides, we set out to model the G $_{aq}$ structure based on the crystal structural coordinates of GTP γ S–G $_{at}$ [3] (see section 2). When assessed for accuracy using the Profile method of Lüthy et al. [39], our G $_{aq}$ computer model (Fig. 4) displayed a 3D–1D mean score of 0.53. This is close to the score established for the G $_{at}$ structure (0.56) indicating that no major folding errors were detectable. This is as



Fig. 4. Computer homology model of $G_{\alpha q}$ structure based on GTP γ S-bound $G_{\alpha i}$. The $G_{\alpha q}$ model is depicted by a grey ribbon (Carson, M. (1990) J. Appl. Cryst. 24, 958–961) while the bound GTP γ S is shown in magenta. The three regions which conformation changes upon GTP binding are shown in blue and are called switch I to III (Lambright, D.G., Noel, J.P., Hamm, H.E. and Sigler, P.B. (1994) Nature 369, 621–628). The two synthetic peptides with the maximal inhibitory activity $G_{\alpha q}$ -stimulated PLC β 1 are shown in red (α 3 helix: residues 252 to 266) and yellow (α 4 helix: residues 306 and 319). Both helices are located in the ras-homology domain.

expected as $G_{\alpha q}$ is 52% identical to $G_{\alpha i}$ and should therefore share a high degree of structural resemblance. This is illustrated by another G-protein α -chain, $G_{\alpha i1}$, which displays a three-dimensional conformation very similar to that of the $G_{\alpha i}$ crystal structure including the position of surface α -helices and loop structures [3–6]. Using our model we have mapped the position of the two inhibitory peptides and Fig. 4 shows their relative position within the $G_{\alpha q}$ structure. Interestingly, both peptides are located within the *ras*-like domain with $G_{\alpha q}$: 251–265 and $G_{\alpha q}$: 306–319 corresponding to helices α 3 and α 4, respectively (nomenclature according to [3]). These two helices are adjacent to one another, and moreover, are localized at the molecular surface of $G_{\alpha q}$ thereby providing a broad surface for interaction with PLC β 1.

The combination of functional data with inhibitory peptides together with homology modelling of $G_{\alpha q}$ suggests an important role for helices α 3 and α 4 in mediating effector interaction.

Such a conclusion is consistent with functional studies performed with $G_{\alpha i}$ where peptides corresponding to these regions mediate activation of cyclic GMP phosphodiesterase through binding inhibitory regulatory γ -subunits [26,27]. Additional studies mapping sites of effector interaction have also been performed for $G_{\alpha s}$ where scanning mutagenesis has identified 4 domains important for activation of adenylyl cyclase [29]. Based on sequence alignments two of these also map to the α 3 and α 4 effector binding helices identified within $G_{\alpha i}$ [26,27] and $G_{\alpha q}$ (this study). It is of note that despite high spatial conservation of helices α 3 and α 4 within the three-dimensional structures of $G_{\alpha i}$, $G_{\alpha s}$ and apparently also $G_{\alpha q}$, the primary sequences in these regions are highly divergent and this may contribute to G-protein specificity of interaction with different effector and second messenger systems. Consistent with this, G-protein α -chains coupling to common effector and second messenger systems show high conservation of primary se-

Table 1

Alignment of primary amino sequences from a subset of G-protein α -chains within the regions corresponding to inhibitory peptides $G_{\alpha q}$: 251–265 and $G_{\alpha q}$: 306–319

Subunit	$G_{\alpha q}$ inhibitory peptides and aligned sequences		Effector Coupling
	$G_{\alpha q}$: 251–265 ($\alpha 3$ – $\alpha 3/\beta 5$)	$G_{\alpha q}$: 306–319 ($\alpha 4$ – $\alpha 4/\beta 6$)	
$G_{\alpha q}$	SKALFRTIITYPWFQ	AREFILKMFVDLNP	\uparrow PLC β
$G_{\alpha 11}$	SKALFRTIITYPWFQ	AREFILKMFVDLNP	\uparrow PLC β
$G_{\alpha 14}$	SKALFRTIITYPWFQ	ARDFILKLYQDQNP	\uparrow PLC β
$G_{\alpha s}$	ALNLFKSIWNNRWLR	AKYFIRDEFRLISTA	\uparrow AC
$G_{\alpha olf}$	SLDLFESIWNNRWLR	AKFFIRDLFLRISTA	\uparrow AC
$G_{\alpha i1}$	SLHLFNSICNHRIFA	.GNYIKVQFLELNMR	\uparrow cGMP-PDE
$G_{\alpha o}$	SLKLFDSICNNKFFI	.AAYIQAQF.ESKNR	\downarrow AC/VDCC
$G_{\alpha i1}$	SMKLFDSICNNKWF	.AAYIQCFEDLNKR	\downarrow AC
$G_{\alpha i2}$	SMKLFDSICNNKWF	.ASYIQSKFEDLNKR	\downarrow AC
$G_{\alpha i3}$	SMKLFDSICNNKWF	.AAYIQCFEDLNRR	\downarrow AC
$G_{\alpha z}$	SLRLFDSICNNWFI	.AVYIQRQFEDLNRR	\downarrow AC

Subunits are grouped according to known regulation of major downstream effector targets. Nomenclature of structural helices (shown in parenthesis) corresponding to peptide sequences are according to [3]. Effector targets are: β isoforms of phospholipase C (PLC β), adenylyl cyclase (AC), retinal cyclic GMP-phosphodiesterase (cGMP-PDE) and voltage-dependent Ca^{2+} channels (VDCC). G-protein coupling was demonstrated by either recombinant expression of selected components or use of antibodies, peptides or antisense oligonucleotides to block endogenous G-protein function (see text for details).

quence within the two regions defined by the inhibitory peptides $G_{\alpha q}$: 251–265 and $G_{\alpha q}$: 306–319 (Table 1). These include activation of PLC $\beta 1$ by $G_{\alpha q}$, $G_{\alpha 11}$ and $G_{\alpha 14}$ [22–24,40,42,43], stimulation of cyclicGMP-phosphodiesterase by $G_{\alpha i1}$ [44], and regulation of adenylyl cyclase by $G_{\alpha s}$ [45,46], $G_{\alpha olf}$ [47], $G_{\alpha i1}$ [48,49] and $G_{\alpha z}$ [48]. $G_{\alpha i1}$ and $G_{\alpha o}$ also play a role regulating Ca^{2+} channel activity [50–52]. As anticipated from such a subclassification, functionally distinct G-proteins display considerably less homology within both regions.

In summary, we report the use of a series of synthetic peptides based on overlapping regions from the entire sequence of $G_{\alpha q}$ to localize sites critical for $G_{\alpha q}$ -dependent activation of its downstream effector PLC $\beta 1$. We observed two clusters of blocking peptides mapping to two distinct domains and represented by $G_{\alpha q}$: 251–265 and $G_{\alpha q}$: 306–319. Computer modelling of $G_{\alpha q}$ reveals that inhibitory peptide sequences form two parallel α -helices lying at the surface of $G_{\alpha q}$. These observations provide the first description of two regions within $G_{\alpha q}$ critically important for activating PLC $\beta 1$, and moreover, indicate that effector binding domains identified in transducin and $G_{\alpha s}$ are conserved spatially in $G_{\alpha q}$.

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