The Amino-terminal Domain of G-protein-coupled Receptor Kinase 2 Is a Regulatory G\(\beta\gamma\) Binding Site

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The amino-terminal domain of G-protein-coupled receptor kinase 2 (GRK2) is activated by free G\(\beta\gamma\) subunits. A G\(\beta\gamma\) binding site of GRK2 is localized in the carboxyl-terminal pleckstrin homology domain. This G\(\beta\gamma\) binding site of GRK2 also regulates G\(\beta\gamma\)-stimulated signaling by sequestering free G\(\beta\gamma\) subunits. We report here that truncation of the carboxyl-terminal G\(\beta\gamma\) binding site of GRK2 did not abolish the G\(\beta\gamma\) regulatory activity of GRK2 as determined by the inhibition of a G\(\beta\gamma\)-stimulated increase in inositol phosphates in cells. This finding suggested the presence of a second G\(\beta\gamma\) binding site in GRK2. And indeed, the amino terminus of GRK2 (GRK2\(1–185\)) inhibited a G\(\beta\gamma\)-stimulated inositol phosphate signal in cells, purified GRK2\(1–185\) bound directly to purified G\(\beta\gamma\) subunits. The amino-terminal G\(\beta\gamma\) regulatory site does not overlap with the RG3 domain of GRK-2 because GRK2\(1–53\), which contains an RG3 domain, did not inhibit G\(\beta\gamma\)-mediated signaling with similar potency and efficacy as did GRK2\(1–185\). In addition to the G\(\beta\gamma\) regulatory activity, the amino-terminal G\(\beta\gamma\) binding site of GRK2 affects the kinase activity of GRK2 because antibodies specifically cross-reacting with the amino terminus of GRK2 suppressed the GRK2-dependent phosphorylation of rhodopsin. The antibody-mediated inhibition was released by purified G\(\beta\gamma\) subunits, strongly suggesting that G\(\beta\gamma\) binding to the amino terminus of GRK2 enhances the kinase activity toward rhodopsin. Thus, the amino-terminal domain of GRK2 is a previously unrecognized G\(\beta\gamma\) binding site that regulates GRK2-mediated receptor phosphorylation and inhibits G\(\beta\gamma\)-stimulated signaling.

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Activated G-protein-coupled receptors are switched off by phosphorylation through G-protein-coupled receptor kinases (GRKs)\(^1\). GRKs are modular proteins consisting of at least three structural domains with different functions. The core kinase domain of GRK2 and GRK3, which regulates the \(\beta\)-adrenergic receptor kinase isozymes, is flanked by an amino-terminal domain, which contains an RG3 domain, and a carboxyl-terminal domain, which contains a pleckstrin homology domain (PH domain) (2–4). The activation of GRK2 and GRK3 requires the activation and dissociation of a heterotrimeric G-protein, i.e. the kinases are activated by free G\(\beta\gamma\) subunits (5, 6). A G\(\beta\gamma\) binding site of GRK2 and GRK3 is localized in the carboxyl terminus of the kinase and overlaps the PH domain (7). Truncation of the PH domain of GRK2 generates a kinase with compromised regulation by G\(\beta\gamma\) subunits (7). The carboxyl-terminal G\(\beta\gamma\) binding site of GRK2 also regulates G\(\beta\gamma\)-stimulated signaling by sequestering free G\(\beta\gamma\) subunits (8). Analyzing the G\(\beta\gamma\) regulatory activity of proteins is a means of identifying G\(\beta\gamma\)-binding proteins or localizing G\(\beta\gamma\) binding sites of proteins (9–11). To find out whether the G\(\beta\gamma\) regulatory activity of GRK2 resides entirely in the carboxyl-terminal PH domain, we analyzed the G\(\beta\gamma\)-sequestering activity of wild-type GRK2 and of carboxyl-terminal-truncated GRK2 mutants. The capacity of those proteins to inhibit a G\(\beta\gamma\)-stimulated increase in inositol phosphates mediated by activation of phospholipase C\(\beta\) was determined (12). We report here that truncation of the carboxyl-terminal G\(\beta\gamma\) binding site of GRK2 did not abolish the G\(\beta\gamma\) regulatory activity of GRK2. A previously unrecognized G\(\beta\gamma\) binding site of GRK2 was identified in the amino terminus of GRK2 that enhances the kinase activity of GRK2 toward the receptor substrate rhodopsin and which inhibits G\(\beta\gamma\)-stimulated signaling.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Cell Transfection—**Human embryonic kidney cells (HEK-293) were cultured and transfected as described previously (13) with plasmids encoding human GRK2 or the indicated truncation mutants of GRK2 or GRK5. The mutants were generated by polymerase chain reaction and were sequenced entirely to confirm the identity of the mutants.

**Determination of Cellular Inositol Phosphate Levels—**Total inositol phosphate levels of HEK-293 cells were determined as described (13). For determination of the G\(\beta\gamma\) regulatory activity of wild-type GRK2 and of the different truncation mutants, cells were co-transfected with plasmids encoding the indicated GRK2 mutants and phospholipase C\(\beta\), G\(\beta\), and G\(\gamma\) (11).

**Protein Purification of GRK2—**Human GRK2 was expressed in Sf9 cells using a recombinant baculovirus. GRK2 was purified by SP-Sepharose and heparin-Sepharose according to established protocols (14, 15). The amino-terminal domain of GRK2 (GRK2\(1–185\)), GRK2\(1–53\), and GRK5\(1–208\) were expressed in *Escherichia coli* as glutathione S-transferase (GST) fusion proteins and purified by affinity chromatography on glutathione-Sepharose 4B according to the manufacturer’s protocol (Amersham Biosciences).

**Phosphorylation of Rhodopsin by GRK2—**The kinase activity of GRK2 was assessed by phosphorylation of the receptor substrate rhodopsin in a total volume of 50 \(\mu\)l of buffer (20 \(mM\) HEPES, pH 7.4) containing 20 \(nm\) GRK2, G\(\beta\gamma\) subunits as indicated, 400 \(nm\) rhodopsin, 10 \(mm\) MgCl\(_2\), 2 \(mm\) EDTA, and 50 \(\mu\)M \([\gamma^32P]ATP\). Phosphorylation was initiated by light and proceeded for 20 min at room temperature. After SDS-PAGE, receptor phosphorylation was assessed by autoradiography. Rhodopsin-enriched membranes were prepared from dark-adapted bovine retinae by sucrose gradient centrifugation (16).

To determine the activation of GRK2 by G\(\beta\gamma\) subunits, various concentrations of purified G\(\beta\gamma\) subunits from bovine brain were incubated

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The abbreviations used are: GRK, G-protein-coupled receptor kinase 2; HEK-293 cells, human embryonic kidney cells; PH domain, pleckstrin homology domain; GST, glutathione S-transferase.

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in the phosphorylation mixture (16). To assess the effect of antibodies specifically cross-reacting with the amino terminus or with the carboxyl terminus of GRK2, polyclonal anti-GRK2 antibodies were immuno-selected by affinity chromatography on GRK2<sub>1-558</sub> or on GRK2<sub>561-689</sub>, respectively, covalently coupled to Affi-Gel-10 (15). The purified antibodies were incubated in the phosphorylation mixture as indicated. Specificity and cross-reactivity of the antibodies with GRK2<sub>1-558</sub> or with GRK2<sub>561-689</sub> were analyzed in immunoblot.

**Immunoblot Detection of Proteins**—Proteins were separated on SDS-containing polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and identified in immunoblot similarly as described (17). Antibodies specific for GRK2 or for G<sub>β</sub> have been characterized previously (14, 18).

**Binding of GRK2<sub>1-282</sub> to G<sub>βγ</sub>**—Purified GST-GRK2<sub>1-160</sub> (100 nM) coupled to glutathione-Sepharose was incubated with G<sub>βγ</sub> subunits (3 nM) in a total volume of 500 μl of buffer (100 mM NaCl, 20 mM HEPES, pH 7.4). After extensive washing with the same buffer, bound G<sub>βγ</sub> subunits were eluted with SDS sample buffer, separated by SDS-PAGE, and identified in immunoblot with G<sub>β</sub>-specific antibodies. As a control, GST-GRK5<sub>1-200</sub> was used instead of GST-GRK2<sub>1-160</sub>.

**ADP-ribosylation of G<sub>α</sub>**—The G<sub>βγ</sub>-mediated enhancement of the pertussis toxin-catalyzed ADP-ribosylation of G<sub>α</sub> was performed with 8 nM G<sub>αs</sub> and 12 nM G<sub>βγ</sub> in the absence or presence of increasing concentrations (10 nM–3 μM) of GST-GRK2<sub>1-165</sub>, GST-GRK2<sub>1-53</sub>, or GST-GRK2<sub>54-165</sub> similarly as described previously (19).

**RESULTS**

**G<sub>βγ</sub> Regulatory Activity of a GRK2 Mutant with Truncated Carboxy-terminal PH Domain**—The carboxy-terminal PH domain of GRK2 is essential for the G<sub>βγ</sub>-dependent phosphorylation of receptor substrates by GRK2 (7). Furthermore, PH domains can regulate G<sub>βγ</sub> signaling by sequestering free G<sub>βγ</sub> subunits (9). To analyze whether the PH domain of GRK2 is entirely responsible for the G<sub>βγ</sub> regulatory activity of GRK2, the PH domain of GRK2 was truncated, and the G<sub>βγ</sub> regulatory activity of the truncated GRK2 mutant (GRK2<sub>1-558CVLL</sub>) was analyzed in intact cells by determining

**Fig. 1.** Truncation of the PH domain of GRK2. **A**, expression of GRK2 (lane 1), GRK2<sub>1-558CVLL</sub> (lane 2), or GRK2<sub>561-689</sub> (lane 3) in HEK-293 cells as determined in immunoblot (IB) with GRK2-specific antibodies. **B**, inositol phosphate levels of cells expressing phospholipase C<sub>β</sub>, G<sub>βγ</sub>, G<sub>γ</sub> (column c, 8–10-fold stimulation of basal), and of cells coexpressing GRK2 (column 1), GRK2<sub>561-689</sub> (column 2), or GRK2<sub>525-689</sub> (column 3). A topology model of GRK2 depicts the localization of the different GRK2 proteins. Data ± S.E. are the means (n = 8).

the inhibition of a G<sub>βγ</sub>-stimulated increase in inositol phosphates mediated by activation of phospholipase C<sub>β</sub> (11, 12). To exclude that a cytosolic localization of the truncated GRK2 mutant prevented the interaction with membrane-anchored G<sub>βγ</sub> subunits in cells, a membrane-anchoring CAXX motif was introduced in GRK2<sub>558CVLL</sub>, similarly as described (7). Wild-type GRK2, the carboxy-terminal-truncated mutant GRK2<sub>1-558CVLL</sub>, and the carboxyl terminus of GRK2 containing the PH domain, GRK2<sub>561-689</sub> were expressed in HEK-293 cells (Fig. 1A, lanes 1–3) and analyzed for their G<sub>βγ</sub> regulatory activity (Fig. 1B). Wild-type GRK2 and GRK2<sub>1-558CVLL</sub> inhibited the G<sub>βγ</sub>-stimulated increase in inositol phosphates (Fig. 1B, columns 1 and 2 versus c). GRK2<sub>561-689</sub> comprising the PH domain of GRK2 also significantly decreased the G<sub>βγ</sub>-stimulated signal (Fig. 1B, column 3). Expression levels of G<sub>βγ</sub> and of phospholipase C<sub>β</sub> were similar in the different experiments (not shown). Together, these data demonstrate that the PH domain of GRK2 inhibits G<sub>βγ</sub>-stimulated signaling in cells but that the G<sub>βγ</sub> regulatory activity of GRK2 is not entirely mediated by the carboxy-terminal PH domain.

**G<sub>βγ</sub> Regulatory Activity of GRK2<sub>1-485</sub>**—To identify the additional domain involved in the G<sub>βγ</sub> regulatory activity of GRK2, GRK2<sub>1-558CVLL</sub> was further truncated. In GRK2<sub>1-485</sub> the entire carboxy-terminal G<sub>βγ</sub> binding site of GRK2 was truncated (7). Wild-type GRK2 and GRK2<sub>1-485</sub> were expressed in HEK-293 cells (Fig. 2A, lanes 1 and 2). Full-length GRK2 (Fig. 2B, column 1) and the truncated mutant GRK2<sub>1-485</sub> significantly inhibited the G<sub>βγ</sub>-stimulated increase in inositol phosphates (Fig. 2B, column 2 versus column c). The inhibition of the G<sub>βγ</sub>-stimulated signal was not dependent on the introduction of a membrane-anchoring CAXX motif in GRK2<sub>1-485CVLL</sub> (Fig. 2, A and B, lane 3, column 3). The carboxy-terminal G<sub>βγ</sub>
The amino-terminal domain of GRK2 regulates Gβγ-stimulated signaling in cells. Expression of GRK2Δ1-185 or of GRK2Δ1-185CVLL in HEK-293 cells suppressed the Gβγ-stimulated increase in inositol phosphate generation. Upper panel, immunoblot (IB) detection of GRK2Δ1-185 (lane 1) and of GRK2Δ1-185CVLL (lane 2) by anti-GRK2 antibodies. Lower panel, inositol phosphate levels of cells expressing phospholipase Cβ1, Gβγ, and Gγ (column c) and of cells coexpressing GRK2Δ1-185 (column 1) or GRK2Δ1-185CVLL (column 2). B, immunoblot of cells expressing GRK5Δ1-100 (lane 1) or GRK5Δ1-200CVLL (lane 2) as detected by anti-GRK5 antibodies and of cells expressing GRK2Δ1-185 (lane 3) detected by anti-GRK2 antibodies (upper panel). The anti-GRK5 and anti-GRK2 antibodies were standardized with purified GRK5 or GRK2, respectively, to produce a signal of equal intensity in immunoblot with equimolar amounts of GRK protein.

The Amino-terminal Domain of GRK2 Regulates Gβγ-stimulated Signaling—In search for the second Gβγ binding site of GRK2, the Gβγ regulatory effect of the amino-terminal domain of GRK2 was analyzed. GRK2Δ1-185 was expressed in HEK-293 cells (Fig. 3A, upper panel, lane 1). GRK2Δ1-185 inhibited the Gβγ-stimulated signal similarly to GRK2Δ1-485, confirming the Gβγ regulatory capacity of the carboxyl-terminal Gβγ binding site of GRK2 (Fig. 2B, column 4). Together, these findings demonstrate that truncation of the carboxyl-terminal Gβγ binding site of GRK2 does not abolish the Gβγ regulatory activity of this kinase, suggesting that GRK2 contains a previously unrecognized Gβγ binding site in addition to the carboxyl-terminal site.

To further confirm that the amino-terminal domain of GRK2 regulates a Gβγ-stimulated signal in cells and in vitro, we asked whether purified GRK2Δ1-185 interacted with Gβγ subunits directly. GRK2Δ1-185 was purified as a GST fusion protein and tested for the inhibition of Gβγ-stimulated rhodopsin phosphorylation. Purified GST-GRK2Δ1-185 inhibited the GRK2-mediated phosphorylation of rhodopsin stimulated by 30 nM Gβγ (IC50: 340 ± 30 nM, Fig. 4A).

GST as a control did not affect the phosphorylation of rhodopsin at concentrations <10 μM (not shown). These findings demonstrate that the amino-terminal domain of GRK2 regulates a Gβγ-stimulated signal in intact cells.
The amino-terminal Gβγ binding domain of GRK2 regulates the kinase activity. A, phosphorylation of rhodopsin (Rh) by 20 nM GRK2 in the presence of increasing concentrations of Gβγ subunits (lane 1, 13 nM; lane 2, 20 nM; lane 3, 40 nM; lane 4, 110 nM; lane 5, 300 nM; lane 6, control, 1 μM; lane 7, 3 μM). B, effect of 100 nM immunoselected antibodies specifically cross-reacting with the amino terminus of GRK2 on the GRK2-mediated phosphorylation of rhodopsin determined as in A in the presence of increasing concentrations of purified Gβγ subunits (lanes 1–7). Data ± S.E. are the means (n = 3).

The Amino-terminal Gβγ Binding Domain of GRK2 Regulates Kinase Activity—The carboxyl-terminal Gβγ binding domain of GRK2 is essential for the Gβγ-dependent stimulation of the kinase activity toward receptor substrates (7). Does the amino-terminal Gβγ binding domain also affect the kinase activity of GRK2? To determine the effect of the amino terminus on the kinase activity, the amino-terminal Gβγ binding domain of GRK2 was targeted with immunoselected antibodies. The purified polyclonal antibodies used for this experiment specifically cross-reacted with GRK21–185 but did not interact with the carboxyl-terminal Gβγ binding domain of GRK2 as determined in immunoblot (not shown). The presence of 100 nM antibodies to the amino terminus of GRK2 suppressed the Gβγ-stimulated (10–40 nM) phosphorylation of rhodopsin by 20 nm GRK2 (Fig. 5, B, lanes 1–3, versus A, lanes 1–3), suggesting that the amino terminus of GRK2 is involved in phosphorylating rhodopsin.

To analyze whether the antibodies interfered with the binding of Gβγ subunits to the amino terminus of GRK2, the concentration of the purified Gβγ subunits was increased (Fig. 5, A and B, lanes 4–7). Gβγ stimulated the phosphorylation of rhodopsin by GRK2 in the absence of antibodies (EC50 = 38 ± 7 nM, Fig. 5A). The presence of 100 nM antibodies specifically cross-reacting with the amino terminus of GRK2 increased the EC50 value of Gβγ in stimulating GRK2-mediated rhodopsin phosphorylation more than 20-fold (Fig. 5, B versus A), but the Gβγ subunits were capable of reversing the antibody-mediated inhibition of the GRK2-induced rhodopsin phosphorylation (Fig. 5B). A higher concentration of the antibodies (250 nM) further increased the EC50 value of Gβγ in stimulating GRK2 (not shown). As a control, unrelated antibodies not cross-reactive with GRK2 did not affect the phosphorylation of rhodopsin by GRK2 (not shown). Together these findings provide strong evidence that the antibodies compete with Gβγ subunits for binding to the amino terminus of GRK2, thereby preventing the stimulatory interaction of Gβγ with the amino terminus.
The interaction of Gβγ with the amino-terminal Gβγ binding domain of GRK2 may thus contribute to the Gβγ-dependence of GRK2.

**Differentiation of the Amino- and Carboxyl-terminal Gβγ Binding Sites of GRK2**—To differentiate between the amino- and carboxyl-terminal Gβγ binding sites of GRK2, the effect of domain-specific antibodies to the carboxyl terminus was assessed in the rhodopsin phosphorylation assay. Antibodies specifically cross-reacting with the carboxyl-terminal domain, GRK2<sup>254–185</sup> (anti-C), inhibited the stimulatory effect of Gβγ at concentrations ranging from 20 nM to 1 μM (Fig. 6A). Interestingly, these antibodies did not alter the GRK2-mediated rhodopsin phosphorylation in the presence of less than 20 nM Gβγ as did the antibodies cross-reacting with the amino terminus (Fig. 6A versus Fig. 5B). Considering that these low Gβγ concentrations in the rhodopsin phosphorylation assay are achieved without the addition of purified Gβγ because the Gβγ subunits come from the rhodopsin-enriched membranes as determined in immunoblot (not shown), this finding is in good agreement with previous observations; a GRK2 mutant lacking the carboxyl-terminal Gβγ binding site is capable of phospho-

rately rhodopsin but lacks the Gβγ-enhancing effect exerted by the addition of purified Gβγ subunits (7). Together these data indicate that the amino- and carboxyl-terminal Gβγ binding sites of GRK2 are functionally different.

The GRK2 activity that was not blocked by the carboxyl-
terminal-specific antibodies was still Gβγ-dependent because increasing concentrations of Gβγ-specific antibodies used as Gβγ scavenger inhibited the residual rhodopsin phosphorylation entirely (Fig. 6B). Similar results were obtained with several other Gβγ-binding proteins such as G<sub>a</sub><sub>o</sub>, or the Raf kinase (not shown). Because the carboxyl-terminal-specific antibodies did not interfere with the Gβγ-binding site (Fig. 6B), these findings reveal again the second Gβγ binding site in GRK2, which is distinct from the carboxyl-terminal site (Fig. 6B). For comparison, antibodies to the amino terminus of GRK2 inhibited the GRK2-mediated rhodopsin phosphorylation under similar conditions in a concentration-dependent manner (Fig. 6C). As controls, the antibodies to the amino terminus of GRK2 did not bind Gβγ (not shown), and carboxyl-terminal antibodies did not interfere with the inhibition exerted by the amino-terminal-specific antibodies (Fig. 6C). Thus, the second Gβγ binding site in the amino terminus of GRK2 is functionally different from the carboxyl-terminal site and is involved in rhodopsin phosphorylation at low concentrations of Gβγ (~20 nM).

**The RGS Domain of GRK2 Does Not Interfere with Gβγ Binding**—The amino terminus of GRK2 contains a previously identified RGS domain (Ref. 2 and 3 and Fig. 7D). The RGS domain overlap with the amino-terminal Gβγ binding site, GRK2<sup>254–185</sup> (Fig. 7A, upper panel versus Fig. 4A), the RGS domain, GRK2<sup>254–185</sup>, had no significant effect when applied at similar concentrations (Fig. 7A, lower panel). This finding strongly suggests that the RGS domain and the amino-terminal Gβγ regulatory site of GRK2 do not overlap. In addition to the Gβγ binding site, GRK2<sup>254–185</sup> contains other regulatory sites such as a receptor interacting site (21) or a calmodulin binding site (22). To exclude the possibility that GRK2<sup>254–185</sup> interfered with the kinase activity of GRK2 in a Gβγ-independent manner, we analyzed the Gβγ regulatory effects of this protein in another Gβγ-dependent assay, the Gβγ-mediated enhancement of the pertussis toxin-catalyzed ADP-ribosylation of Go<sub>a</sub> (19). GRK2<sup>254–185</sup> inhibited the enhancing effect of Gβγ on the ADP-ribosylation of Go<sub>a</sub> (Fig. 7B) with similar potency and efficacy as did GRK2<sup>21–53</sup> (IC<sub>50</sub> = 290 ± 20 and 360 ± 30 nM of GRK2<sup>254–185</sup> and GRK2<sup>21–53</sup>, respectively). In contrast, the RGS domain, GRK2<sup>254–185</sup>, had no significant effect at concentrations <1 μM (Fig. 7B). Thus, GRK2<sup>254–185</sup> encompasses the functionally important portion of the amino-terminal Gβγ regulatory site of GRK2, whereas the RGS domain of GRK2, GRK2<sup>254–185</sup>, did not interfere significantly with Gβγ binding. The *in vitro* findings were confirmed in cells. Although GRK2<sup>254–185</sup> inhibited Gβγ-stimulated signaling similarly to GRK2<sup>21–53</sup>, the RGS domain GRK2<sup>254–185</sup> did not affect the Gβγ-stimulated increase in inositol phosphates mediated by PLC-β<sub>1</sub> (Fig. 7C, first through fourth columns). By contrast, the
RGs domain-containing GRK2\textsuperscript{1–185} and GRK2\textsuperscript{24–185} efficiently inhibited a Go\textsubscript{a}–stimulated inositol phosphate signal mediated by PLC-\(\beta_1\) that was not affected by GRK2\textsuperscript{1–50} (Fig. 7C, fifth through eighth columns).

**DISCUSSION**

The kinase activity of GRK2 and GRK3 toward receptor substrates is strongly enhanced by G\(\beta\gamma\) subunits. The G\(\beta\gamma\) dependence links the kinase activity of these GRKs to the activation of a heterotrimeric G-protein. A carboxyl-terminal PH domain in GRK2 and GRK3 is essentially involved in the G\(\beta\gamma\) dependence of GRKs (7). Here we present strong evidence that the amino-terminal domain of GRK2 contains a second G\(\beta\gamma\) binding site that contributes to the regulation of GRK2 by low concentrations of G\(\beta\gamma\) subunits; (i) the amino terminus of GRK2 inhibited G\(\beta\gamma\)-stimulated signaling in cells and *in vitro*, (ii) GRK2\textsuperscript{1–185} interacts directly with purified G\(\beta\gamma\) subunits, (iii) targeting of the amino-terminal G\(\beta\gamma\) binding domain of GRK2 by site-directed antibodies suppressed the GRK2-mediated phosphorylation of rhodopsin, and (iv) this inhibition was released by an excess of free G\(\beta\gamma\) subunits.

The amino terminus of GRK2 contains several important structural elements, an RGS-domain affecting Go\textsubscript{a}–stimulated signaling (2, 3), a calmodulin binding site (22), which is regulated by protein kinase C phosphorylation (23), and a receptor interacting site (21). A receptor interacting site and a calmodulin binding site were also localized in the carboxyl-terminal domain of GRK2 (22, 24). These functional similarities of the amino- and the carboxyl-terminal domains of GRK2 are complemented by the localization of a previously unrecognized G\(\beta\gamma\) binding site in the amino terminus of GRK2. The novel amino-terminal G\(\beta\gamma\) binding site is involved in the G\(\beta\gamma\) dependence of GRK2 in addition to the carboxyl terminus. A topological model of GRK2 appears to consist of a core kinase domain flanked by two structurally and functionally different G\(\beta\gamma\) binding domains. With two G\(\beta\gamma\) binding sites, GRK2 activity is tightly controlled by G\(\beta\gamma\) subunits over a wide concentration range. Thereby G\(\beta\gamma\) subunits translate the intensity of a G-protein-stimulated signal into GRK2 activity to switch off the signal-generating receptor.

Apart from the functional importance of the newly identified G\(\beta\gamma\) binding site in the amino terminus of GRK2, this domain may constitute a novel target allowing the selective inhibition of GRK2-mediated receptor phosphorylation by pharmacological tools. Site-directed antibodies to the kinase amino terminus suppressed the phosphorylation of rhodopsin by GRK2. Because such an inhibition was released by the addition of an excess of G\(\beta\gamma\) subunits, blockade of GRK2 activity by pharmacological compounds binding to the amino terminus of GRK2 would be reversed upon excessive G-protein activation, i.e. G\(\beta\gamma\) release. The proposed mechanism could allow the design of fine-tuning GRK inhibitors, which would amplify low threshold signals and maintain desensitization of excessive stimuli. Additional experiments will have to identify such compounds to validate the proposed principle under physiological conditions.

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