Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Current Opinion in
Cell Biology

Structural insights into G protein-coupled receptor kinase function

Kristoff T Homan and John JG Tesmer

The atomic structure of a protein can greatly advance our understanding of molecular recognition and catalysis, properties of fundamental importance in signal transduction. However, a single structure is incapable of fully describing how a protein functions, particularly when allostery is involved. Recent advances in the structure and function of G protein-coupled receptor (GPCR) kinases (GRKs) have concentrated on the mechanism of their inhibition by small and large molecules. These studies have generated a wealth of new information on the conformational flexibility of these enzymes, which opens new avenues for the development of selective chemical probes and provides deeper insights into the molecular basis for activation of these enzymes by GPCRs and phospholipids

Addresses
Life Sciences Institute and the Departments of Pharmacology and Biological Sciences, University of Michigan, Ann Arbor, MI 48109, United States

Corresponding authors: Tesmer, John JG (tesmerjj@umich.edu)

Current Opinion in Cell Biology 2014, **27**:25–31

This review comes from a themed issue on **Cell regulation**

Edited by **Jeffrey L Benovic** and **Mark von Zastrow**

For a complete overview see the Issue and the Editorial

Available online 26th November 2013

0955-0674/$ – see front matter, © 2013 Elsevier Ltd. All rights reserved.

http://dx.doi.org/10.1016/j.ceb.2013.10.009

Introduction

G protein-coupled receptor (GPCR) kinases (GRKs) initiate the homologous desensitization of activated GPCRs through the phosphorylation of specific sites within the cytoplasmic loops and carboxy-terminal tails of the receptors [1]. These covalent modifications help to recruit arrestins, which uncouple the GPCRs from heterotrimeric G proteins and targets them for internalization. There are 7 mammalian GRKs grouped into 3 subfamilies (GRK1, GRK2, and GRK4) [2] (Figure 1). Atomic structures representing each subfamily (GRK1 [3], GRK2 [4,5], and GRK6 [6,7••]) in various ligand-bound states are now available. These structures establish that the conserved structural core of GRKs is comprised of a protein kinase domain inserted into a loop of a regulator of G protein signaling homology (RH) domain [8]. The RH domain serves as an intramolecular scaffold that maintains the small lobe of the kinase domain in a state that is competent to phosphorylate activated GPCRs. Consequently, the kinase domain, although closely related to those of protein kinases A, G and C (AGC kinases), does not require phosphorylation on its activation loop for full activity. GRKs, however, retain the C-terminal extension of the kinase domain characteristic of the AGC kinase family, which contributes residues to the active site cleft. Although this element is not fully ordered in most GRK structures, mutations in this region in GRK2 [9] and GRK1 [10] are known to dramatically inhibit the phosphorylation of receptor and soluble substrates, consistent with the idea that this element serves to regulate kinase activity as it does in other AGC kinases [11]. The first $\sim$20 amino acids of GRKs are highly conserved and critical for GPCR and phospholipid-stimulated autophosphorylation. However, this region is disordered in most GRK structures reported to date, clouding interpretation of its molecular role.

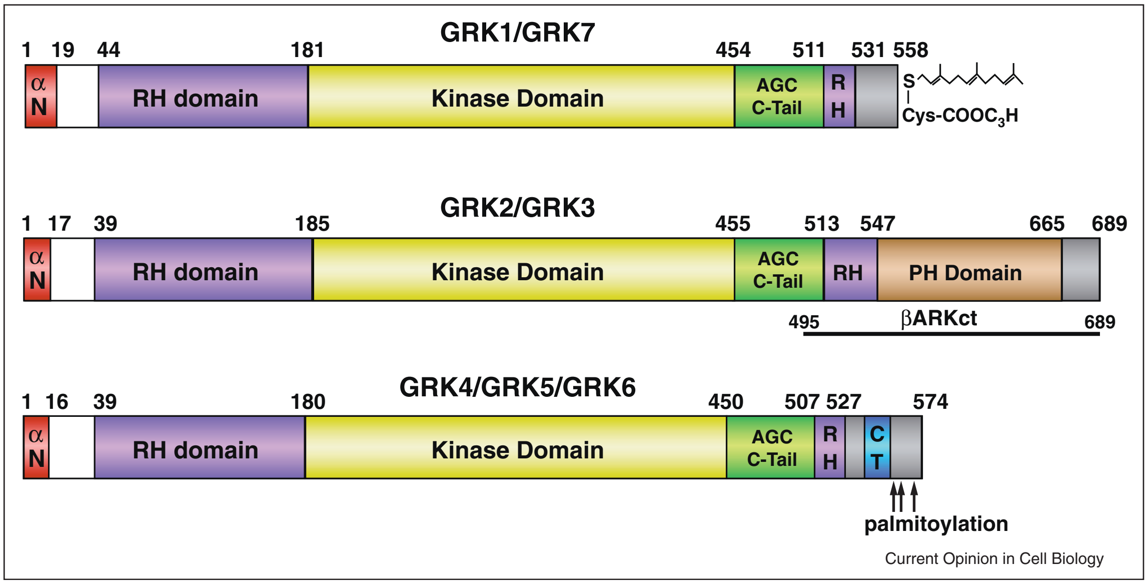
This review highlights recent advances in our molecular understanding of GRK function. The most recent structural studies have emphasized the conformational variability of the GRK kinase domain, an understanding of which will likely be key for the development of selective chemical probes. Some of the observed conformational changes observed have also provided much needed structural insight into how these enzymes are recognized and activated by agonist occupied GPCRs and/or phospholipids.

Inhibiting the GRKs

Various GRKs are known to play roles in human disease [12]. GRK2 and GRK5 stand out due to their well characterized roles in heart failure and cardiac hypertrophy [13–17]. One of the most selective inhibitors of GRK2 known is βARKct (Figure 1), a fragment corresponding to the 222 C-terminal residues of GRK2 [13,18], which can be administered via adeno-associated virus gene delivery and improves contractile performance in both small and large animal models of heart failure [14,19]. This protein serves as a dominant negative because it blocks the recruitment of endogenous GRK2 to the membrane by heterotrimeric Gβγ subunits. Disadvantages of this approach are that all Gβγ signaling pathways would be impacted and humoral immunity may limit effectiveness. Clearly, small cell permeable molecules that can directly and selectively inhibit a specific GRK would be of great use in both the laboratory and clinic.

In the last three years there has been a dramatic expansion of our molecular understanding of how GRKs interact with inhibitors. Crystal structures of GRK2 in complex with an RNA aptamer that inhibits GRK2 with high affinity (3.3 nM) and selectivity (60-fold and 180-fold

Figure 1



Current Opinion in Cell Biology

Domain structure of the three mammalian GRK subfamilies. The αN helix (red) is believed to engage the membrane and/or activated GPCRs. It has also proposed to simultaneously engage the kinase domain (yellow) and the C-terminal kinase extension (green) to stabilize the kinase domain in a more active conformation. The C-terminal region of all GRKs contributes to membrane localization, although in a subfamily-specific manner: it is prenylated in the GRK1 subfamily, it binds to Gβγ subunits in the GRK2 subfamily, and it has a basic amphipathic C-terminal helix (CT) and/or palmitoylation sites in the GRK4 subfamily. A black bar shows the region of GRK2 comprising the βARKct protein.

versus GRK6 and GRK1, respectively) were recently reported [20,21•]. In these structures, a hairpin loop of the aptamer mimics the interactions of ATP in the active site (Figure 2a), and the RNA phosphodiester backbone forms extensive electrostatic interactions that remodels the large lobe of the kinase domain. The latter interaction may emulate how polyanionic macromolecules such as heparin inhibit GRK function [22,23]. The kinase domain of GRK2 in complex with the aptamer adopts an unusually 'open' conformation that is likely enforced by the large size of the RNA hairpin.

More drug-like molecules have also been structurally characterized in complex with GRK2, including the natural product balanol (Figure 2b), which exhibits an IC_{50} of 50 nM for GRK2, an order of magnitude lower than for GRK5 and GRK1 [24]. Remarkably, a series of related compounds developed by Takeda Pharmaceuticals demonstrate both high potency (50–300 nM IC_{50} values) and greater than 2000-fold selectivity for GRK2 over other GRKs [25,26•]. However, balanol is a pan-AGC kinase inhibitor and represents a relatively difficult chemical synthesis, and the Takeda compounds have not progressed to clinic trials. Both classes of molecules consist of a linear arrangement of four ring systems that bind in the active site and trap the kinase domain in conformation that is thought to be the apo state of GRK2. The last ring system of these inhibitors extends into a region called the hydrophobic subsite [27] formed between the small and large lobes of the kinase domain (Figure 2b). A major conclusion from these structures and associated biochemical studies is that selectivity for GRK2 is likely driven more by the conformation of the kinase domain than the identity of residues in the active site, which are highly conserved among GRKs and other AGC kinases. The differential affinity of GRKs for ATP and ADP may also contribute to selectivity in kinetic assays (Homan KT, Wu E, Wilson MW, Singh P, Larsen SD, Tesmer JJG: **Structural and functional analysis of G protein-coupled receptor kinase inhibition by paroxetine and a rationally designed analog**. *Mol Pharmacol*, in press).

Most recently, the selective serotonin reuptake inhibitor paroxetine was identified as a low μM inhibitor of GRK2, with similar selectivity as balanol [28••]. Remarkably, the drug inhibited GRK2-dependent GPCR phosphorylation in living cells and improved heart contractility in live mice. Like balanol and the Takeda compounds, paroxetine binds in a manner that overlaps with the ATP binding site, but it is a shorter molecule with only three ring systems and does not extend into the hydrophobic subsite. Because it exhibits a lower potency of inhibition than balanol and the Takeda compounds, occupation of this subsite may be a perquisite for high-affinity binding. Interestingly, the kinase domain of GRK2 adopts a novel, relatively closed conformation when bound to paroxetine that promotes order in the C-terminal extension of the kinase domain (Figure 2). This

Download English Version:

https://daneshyari.com/en/article/8465953

Download Persian Version:

https://daneshyari.com/article/8465953

Daneshyari.com