Published in final edited form as:

Biochim Biophys Acta. 2013 July ; 1834(7): 1346–1351. doi:10.1016/j.bbapap.2013.02.009.

The Switch Helix: A putative combinatorial relay for interprotomer communication in cGMP-dependent protein kinase

Thomas M. Moon[§], Brent W. Osborne[¶], and Wolfgang R. Dostmann^{§,*}

[§]Department of Pharmacology, The University of Vermont, Burlington, VT 05405

[¶]Department of Physiology, Columbia University Medical Center, New York, NY 10032

Abstract

For over three decades the isozymes of cGMP-dependent protein kinase (PKG) have been studied using an array of biochemical and biophysical techniques. When compared to its closest cousin, cAMP-dependent protein kinase (PKA), these studies revealed a set of identical domain types, yet containing distinct, sequence-specific features. The recently solved structure of the PKG regulatory domain showed the presence of the switch helix (SW), a novel motif that promotes the formation of a domain-swapped dimer in the asymmetric unit. This dimer is mediated by the interaction of a knob motif on the C-terminal locus of the SW, with a hydrophobic nest on the opposing protomer. This nest sits adjacent to the cGMP binding pocket of the B-site. Priming of this site by cGMP may influence the geometry of the hydrophobic nest. Moreover, this unique interaction may have wide implications for the architecture of the inactive and active forms of PKG.

Introduction

All PKG isoforms are essential signaling components in virtually every mammalian tissue. As members of the AGC family of basophilic protein kinases, PKG type I and II isoforms serve as the principle intracellular receptors for the second-messenger cyclic 3',5'-guanosine-monophosphate (cGMP) [1]. In a fashion thought to be similar to PKA activation, cGMP binds to the regulatory domain at two sites to promote activation of the protein kinase [2, 3]. However, several factors challenge a simple description of the structural events that lead to PKG holoenzyme activation, including the order of the high and low-affinity cyclic nucleotide-binding sites, unique structural and biochemical differences in the dimerization domains of PKG and PKA, and the feature that the catalytic domain of PKG is linked to its regulatory domain in the same polypeptide chain.

Remarkably, the type I and type II PKG isoforms share an overall identical domain organization consisting of N-terminal regulatory domains and C-terminal catalytic domains (Figure 1) [4, 5]. Alternative splicing of the type I α and I β isoforms provide the largest deviation from sequence homology [6-8]. Type II PKG results from a different gene

© 2012 Elsevier B.V. All rights reserved.

*Corresponding author. The University of Vermont, College of Medicine, Department of Pharmacology, Burlington, VT 05405, USA. wolfgang.dostmann@uvm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

altogether, and again shows limited homology to type I [9]. Because of these amino acid sequence differences, it has been proposed that residues from the dimerization and autoinhibitory domains are largely responsible for the kinetic phenotype of the PKG isoforms (Figure 2a) [10-12]. Following the autoinhibitory domain, all PKG isoforms are nearly identical in amino acid sequence. First, the regulatory domains carry two in-tandem cGMP binding sites A and B (Figure 2b) [2, 4]. In contrast to PKA, the N-terminal A site is the high affinity binding site and the C-terminal B site the low affinity site in PKG [13, 14]. It has been a particular challenge to understand these compositional differences between PKA and PKG in terms of their contributions to the molecular mechanisms of kinase activation. In addition, the catalytic domain resides C-terminal to the low affinity cGMP-binding site B with minimal sequence overlap and homology to PKA [15].

In an attempt to shed light on the molecular mechanisms of PKG activation, recent crystallographic studies have examined the isolated N-terminal dimerization domain (DD) ($I\beta$, 5-55), the isolated high affinity cGMP binding site ($I\beta$, 92-227) as well as the majority of the regulatory domain ($I\alpha$, 78-355) [16-18]. In PKG, like that of PKA, the N-terminal DD provides the structural-basis for homodimer formation. This alignment has been repeatedly confirmed in both type I isoforms by multiple biophysical techniques; however, the only high-resolution structure available is derived from the $I\beta$ isoform and confirms previous experimental examination of the $I\alpha$ isoform [18-21]. Examination of the latter constructs revealed that the A-site of type I PKG isoforms could bind both cAMP and cGMP, and the identification a previously uncharacterized domain called the switch helix (SW) that promotes dimerization between protomers. The discovery of these unexpected interactions may prove valuable in discerning the molecular events that are crucial for domain reorganization accompanied during PKG holoenzyme activation.

Choosing symmetry-related solutions to the crystal structure of PKG $I\alpha$ (78-355)

The structure of the homodimeric form of the PKG $I\alpha$ regulatory subunit detailed a new paradigm for considering PKG holoenzyme formation [17]. PKG 78-355 crystals diffracted to 2.5 Å and grew in the C2 space group. The asymmetric unit contained two protein molecules related by non-crystallographic symmetry along a 2-fold axis for which electron density was resolved for the entire construct. The orientation of dimerization was chosen by energetic analysis of the two prominent dimers in the asymmetric unit (Figure 3). Independent PDBe/PISA analysis found that, although the *iso* dimer (presented by the two regulatory subunits interacting in an anti-parallel fashion directly via the A- and B-sites, Figure 3A) had an interaction area of 4640 Å² (almost 2-fold greater than the SW model), the free energy of interaction was more favorable for the SW dimer (Figure 3B) [22]. The *iso* dimer had a free energy calculation of -6.6 kcal/mol (ΔG^{int}), requiring 9.6 kcal/mol for dissociation (ΔG^{diss}). The SW model buries 2740 Å² with ΔG^{int} calculated at -26.4 kcal/mol and a ΔG^{diss} of 15.8 kcal/mol. Further investigation of these two dimers examined the *p*-value associated with the interaction free energy. These values suggested the *iso* dimer interface (*p*=0.890) was indicative of an interaction having been formed as a consequence of crystallization, while the SW model (*p*=0.027) likely formed from biologically-relevant contacts. Taken together, this is a strong suggestion that the SW model may represent a physiologically significant macromolecular assembly. The more favorable energetics of the SW model stem from a distinct set of interactions between residues at the C-terminal end of the PKG 78-355, which sit in a nest of hydrophobic residues from the B-site of the opposing symmetry mate. This interaction is further strengthened by a specific hydrogen bond between the backbone carbonyl of T220 and the side chain amino group of N353. No such hydrophobic or specific interactions are observed in the antiparallel orientation of the *iso* dimer model.

The SW-helix as a novel means of allosteric regulation

The structure of the regulatory subunit of PKG I α revealed a unique interaction site at the C-terminal tail of the SW helix motif that we termed the “knob/nest” (Figure 4). The knob motif is located at the C-terminus of the SW helix on the opposing protomer and is composed of the conserved residues FFANL, which is present in both PKG I α and I β , but not PKG II (Figure 4b). The nest site is a sub-domain of the cyclic nucleotide B-site (Figure 4a) and is composed of hydrophobic residues originating from the N3A motif, α B, β 6, and the phosphate-binding cassette (PBC). The nest directly abuts the cGMP binding site and is partly formed by β 6 and the PBC (Figure 5a). The knob/nest interaction in solution was also supported by deuterium exchange mass spectrometry (DxMS) wherein we observed partial protection of C-terminal tail residues that encompassed the knob motif [17]. This further strengthens the putative physiological significance of the SW dimer predicted by PDBe/PISA.

The majority of the interactions provided by the knob motif with the nest residues are between F350, F351 and L354, which encompass 1332 Å² of buried surface area out of a total of 2740 Å² for the SW dimer (Figure 5b). Although the B-site is unoccupied by cyclic nucleotide, when compared to the previously reported A-sites from PKG I β that contain cyclic nucleotide, a structural alignment reveals that there is a 4.8 Å shift in the PBC associated with cGMP binding but only 3.3 Å with cAMP (Figure 6) [16]. This alignment of these three structures found that there was an RMSD <1 Å within the β -barrel of these structures.

The conformational state of the cGMP binding site in the B-domain and its contiguous nature with the hydrophobic nest may suggest an allosteric coupling between these two subdomains. Specifically, movement of the PBC in the B-site may open additional exposed surface area through the sliding of L294 in the PBC away from α B. As a result, this may affect the affinity of the SW knob for the nest and would have the B-site serve as a combinatorial relay for the detection of SW, cGMP, or both.

The cyclic nucleotide binding sites in PKG are discrete

The regulatory domain of cyclic guanosine monophosphate-activated protein kinase contains two sites that are allosterically coupled to aid in activation of the catalytic subunit of PKG [23, 24]. In a fashion similar to cyclic nucleotide binding to PKA, there is a high-affinity and a low-affinity site. In PKG, these sites are the A and B-sites, respectively, unlike PKA, where the order is reversed [13, 14]. Recent crystallographic studies have discovered that the A-site in the regulatory subunit of PKG I isoforms can bind both cAMP and cGMP, although the implications for this are yet unexplored [16]. Specifically, this study demonstrated that the isolated A-site from PKG I β was not as selective against cGMP (12 nM), versus cAMP (27 nM), as previously thought. In the structure of the intact regulatory domain from PKG I α , cAMP is found in the A-site, despite extensive dialysis of the purified protein prior to crystallization [17]. We speculate that the presence of cAMP in the regulatory subunit of PKG I α and the fact that there is little difference in the affinity of cAMP or cGMP to the A-site of PKG I β is not coincidental. Inspection of the nucleotide binding sites in PKG type I isoforms suggest that the placement of an arginine on β 5 within the B-site of the regulatory subunit may provide a mechanism of nucleotide selectivity (**blue arrows** Figure 2). When compared with the B-site, the A-site is more solvent-exposed and does not have a charged residue in the equivalent position. We further hypothesize that the lack of charged residues that would dictate for cAMP or cGMP in the A-site of the PKG regulatory domains might indicate that PKG is a dual-selectivity cyclic nucleotide-activated protein kinase. Since PKG is able to bind cAMP or cGMP with similar affinity in the A-site,

this would act as a priming step within the regulatory domain in the event of elevated cAMP levels. A consequence of this would be such that even a subtle rise in endogenous cGMP levels would cause activation of the signaling cascade. Further, in the absence of cAMP, increasing levels of cGMP would still activate the kinase under normal conditions.

This mechanism is made more palatable by the lack of broad conformational changes in our structure of the PKG regulatory subunit [17]. We did not observe significant compaction of the two nucleotide sites like that of the cAMP-bound structure of PKA:RI α [25]. However, when compared to the cAMP-free structure of the PKA RI α :C holoenzyme complex, the PKG I α regulatory domain appears to have a slight twist in the relative orientation of the A and B-sites in addition to a distortion of the B/C helix spanning the two nucleotide sites (Figure 7). This hypothesis of non-specific nucleotide targeting to the A-site of PKG is also enriched by the placement of R281 along β 5 in the B-site. This arginine, along with K292 in the PBC may act as capping residues for the interaction of the guanine keto-oxygen with the guanidium or amine groups from these residues and provide the mechanism for selectivity in the B-site of the PKG regulatory domain.

The structure of the PKG regulatory subunit containing the SW helix has implications for PKG activation

The energetic contribution provided by the formation of the knob/nest arrangement reported in Osborne *et al.* suggests that the SW helix may play an active role in formation of the catalytically active PKG I α /I β holoenzyme. Mutation of the knob residues to alanine partially abrogates activation of the enzyme upon stimulation with cGMP, consistent with predictions of the physiological significance of the SW dimer [17]. These mutations of the knob residues suggested a new model for holoenzyme complex formation such that the catalytic domain at the C-terminus of PKG would associate with the B-site of the regulatory domain in the opposing protomer. However, examination of PKA heterodimer formation between the catalytic subunit and multiple regulatory isoforms reveal that the PBC in the A-site consistently interacts with the substrate cleft in the catalytic subunit [26-30]. While these observations in PKG I isoforms are accounted for by biochemical studies, they do not, however, adequately account for PKG II activity. In the type II isoform there is the striking lack of conserved knob residues and the disruption of the hydrophobic nest by the replacement of the equivalent I228 to lysine (**red arrow** Figure 4a). The increase in the size of the linker between the regulatory domain and the catalytic subunit in PKG II may be more accommodating to a *syn*-mechanism of activation than those proposed by the formation of the SW dimer due to changes in these two important interaction sites.

These core interactions in PKG I isoforms formed between heterodimers of the catalytic and regulatory subunits appear to be invariant even though quaternary arrangements of PKA holoenzymes differ appreciably [28, 29, 31-33]. If PKG I isoforms maintain similar interactions to those observed in PKA, this would suggest that the structure presented in Osborne, *et al.* would not necessarily represent an inactive conformation. First, the PBC in the B-site is occluded by the interaction of the hydrophobic nest with the knob residues. Inclusive with the previous observation, the PBC in the A-site would be inaccessible due to the low number of residues (seven) spanning the linker region between the SW and the beginning of the catalytic domain of PKG. It is unlikely, given the homology between the regulatory and catalytic domains of PKG and PKA, that an interaction that is conserved across PKA isoforms would be abandoned in PKG, although it is possible. Despite the fact that a crystal structure of the PKG holoenzyme does not exist, we can now begin to construct a model of PKG activation based upon the current cache of available crystallographic data (Figure 8). This model suggests the dimerization promoted by the SW is induced by the binding of cGMP, and subsequent dissociation of the catalytic and

regulatory domains. Moreover, the presence of the SW may help explain the synergistic nature of PKG activation observed in the holoenzyme.

Conclusions

Until recently, the lack of high-resolution structures of PKG has crippled efforts to incorporate the wealth of available low-resolution data into a more comprehensive allosteric model of PKG. A new, and more detailed view of the PKG holoenzyme is now emerging through new sequence and structure analyses. These indicate that the type I isoforms of PKG share domain composition that is unique to this sub-family of cyclic-nucleotide protein kinases and not to the type II isoform. Specifically, a transitional domain found only in the type I isoforms, called the SW helix, bridges between regulatory and catalytic domains and has been shown to be essential for PKG activation. While investigation of this motif has been limited, the contributions of the SW to the collective understanding of the structural similarities and differences within the PKG isozymes reveal features with unforeseen consequences as to holoenzyme assembly and the molecular mechanism of activation.

Acknowledgments

This work was funded by grants from the National Institutes of Health R01 HL068991 (WRD), T32 HL007647 (TMM) and the Totman Trust for Medical Research.

Bibliography

- [1]. Hofmann F, Bernhard D, Lukowski R, Weinmeister P. cGMP regulated protein kinases (cGK). Handbook of experimental pharmacology. 2009;137–162. [PubMed: 19089329]
- [2]. Francis SH, Corbin JD. Cyclic Nucleotide-Dependent Protein Kinases: Intracellular Receptors for cAMP and cGMP Action. Critical Reviews in Clinical Laboratory Sciences. 1999; 36:275–328. [PubMed: 10486703]
- [3]. Francis SH, Corbin JD. Structure and function of cyclic nucleotide-dependent protein kinases. Annu Rev Physiol. 1994; 56:237–272. [PubMed: 8010741]
- [4]. Pfeifer A, Ruth P, Dostmann W, Sausbier M, Klatt P, Hofmann F. Structure and function of cGMP-dependent protein kinases, Reviews of physiology. biochemistry and pharmacology. 1999; 135:105–149.
- [5]. Hofmann, F. The Protein Kinase FactsBook. In: Hardie, GH.; Steven, editors. Protein-Serine Kinases. Academic Press; San Diego, CA: 1995. p. 73-76.
- [6]. Orstavik S, Natarajan V, Tasken K, Jahnsen T, Sandberg M. Characterization of the human gene encoding the type I alpha and type I beta cGMP-dependent protein kinase (PRKG1). Genomics. 1997; 42:311–318. [PubMed: 9192852]
- [7]. Sandberg M, Natarajan V, Ronander I, Kalderon D, Walter U, Lohmann SM, Jahnsen T. Molecular cloning and predicted full-length amino acid sequence of the type I beta isozyme of cGMP-dependent protein kinase from human placenta. Tissue distribution and developmental changes in rat. FEBS Lett. 1989; 255:321–329. [PubMed: 2792381]
- [8]. Wernet W, Flockerzi V, Hofmann F. The cDNA of the two isoforms of bovine cGMP-dependent protein kinase. FEBS Lett. 1989; 251:191–196. [PubMed: 2546820]
- [9]. Gamm DM, Francis SH, Angelotti TP, Corbin JD, Uhler MD. The type II isoform of cGMP-dependent protein kinase is dimeric and possesses regulatory and catalytic properties distinct from the type I isoforms. The Journal of biological chemistry. 1995; 270:27380–27388. [PubMed: 7593002]
- [10]. Ruth P, Pfeifer A, Kamm S, Klatt P, Dostmann WR, Hofmann F. Identification of the amino acid sequences responsible for high affinity activation of cGMP kinase Ialpha. The Journal of biological chemistry. 1997; 272:10522–10528. [PubMed: 9099696]

- [11]. Ruth P, Landgraf W, Keilbach A, May B, Egleme C, Hofmann F. The activation of expressed cGMP-dependent protein kinase isozymes I alpha and I beta is determined by the different amino-termini. *Eur J Biochem.* 1991; 202:1339–1344. [PubMed: 1662612]
- [12]. Richie-Jannetta R, Busch JL, Higgins KA, Corbin JD, Francis SH. Isolated regulatory domains of cGMP-dependent protein kinase Ialpha and Ibeta retain dimerization and native cGMP-binding properties and undergo isoform-specific conformational changes. *The Journal of biological chemistry.* 2006; 281:6977–6984. [PubMed: 16407222]
- [13]. Reed RB, Sandberg M, Jahnsen T, Lohmann SM, Francis SH, Corbin JD. Structural order of the slow and fast intrasubunit cGMP-binding sites of type I alpha cGMP-dependent protein kinase. *Adv Second Messenger Phosphoprotein Res.* 1997; 31:205–217. [PubMed: 9344253]
- [14]. Reed RB, Sandberg M, Jahnsen T, Lohmann SM, Francis SH, Corbin JD. Fast and slow cyclic nucleotide-dissociation sites in cAMP-dependent protein kinase are transposed in type Ibeta cGMP-dependent protein kinase. *The Journal of biological chemistry.* 1996; 271:17570–17575. [PubMed: 8663415]
- [15]. Takio K, Wade RD, Smith SB, Krebs EG, Walsh KA, Titani K. Guanosine cyclic 3',5'-phosphate dependent protein kinase, a chimeric protein homologous with two separate protein families. *Biochemistry.* 1984; 23:4207–4218. [PubMed: 6091741]
- [16]. Kim JJ, Casteel DE, Huang G, Kwon TH, Ren RK, Zwart P, Headd JJ, Brown NG, Chow D-C, Palzkill T, Kim C. Co-Crystal Structures of PKG Iβ (92–227) with cGMP and cAMP Reveal the Molecular Details of Cyclic-Nucleotide Binding. *PLoS ONE.* 2011; 6:e18413. [PubMed: 21526164]
- [17]. Osborne BW, Wu J, McFarland CJ, Nickl CK, Sankaran B, Casteel DE, Woods VL Jr. Kornev AP, Taylor SS, Dostmann WR. Crystal structure of cGMP-dependent protein kinase reveals novel site of interchain communication. *Structure.* 2011; 19:1317–1327. [PubMed: 21893290]
- [18]. Casteel DE, Smith-Nguyen EV, Sankaran B, Roh SH, Pilz RB, Kim C. A crystal structure of the cyclic GMP-dependent protein kinase I{beta} dimerization/docking domain reveals molecular details of isoform-specific anchoring. *The Journal of biological chemistry.* 2010; 285:32684–32688. [PubMed: 20826808]
- [19]. Atkinson RA, Saudek V, Huggins JP, Pelton JT. 1H NMR and circular dichroism studies of the N-terminal domain of cyclic GMP dependent protein kinase: a leucine/isoleucine zipper. *Biochemistry.* 1991; 30:9387–9395. [PubMed: 1892839]
- [20]. Richie-Jannetta R, Francis SH, Corbin JD. Dimerization of cGMP-dependent protein kinase Ibeta is mediated by an extensive amino-terminal leucine zipper motif, and dimerization modulates enzyme function. *The Journal of biological chemistry.* 2003; 278:50070–50079. [PubMed: 12933804]
- [21]. Zweckstetter M, Schnell JR, Chou JJ. Determination of the Packing Mode of the Coiled-Coil Domain of cGMP-Dependent Protein Kinase Iα in Solution Using Charge-Predicted Dipolar Couplings. *Journal of the American Chemical Society.* 2005; 127:11918–11919. [PubMed: 16117512]
- [22]. Krissinel E, Henrick K. Inference of macromolecular assemblies from crystalline state. *Journal of molecular biology.* 2007; 372:774–797. [PubMed: 17681537]
- [23]. McCune RW, Gill GN. Positive cooperativity in guanosine 3':5'-monophosphate binding to guanosine 3':5'-monophosphate-dependent protein kinase. *The Journal of biological chemistry.* 1979; 254:5083–5091. [PubMed: 36381]
- [24]. Corbin JD, Doskeland SO. Studies of two different intrachain cGMP-binding sites of cGMP-dependent protein kinase. *The Journal of biological chemistry.* 1983; 258:11391–11397. [PubMed: 6309846]
- [25]. Su Y, Dostmann W, Herberg F, Durick K, Xuong N, Ten L, Eyck S, Taylor K. Varughese, Regulatory subunit of protein kinase A: structure of deletion mutant with cAMP binding domains. *Science.* 1995; 269:807–813. [PubMed: 7638597]
- [26]. Kim C, Cheng CY, Saldanha SA, Taylor SS. PKA-I Holoenzyme Structure Reveals a Mechanism for cAMP-Dependent Activation. *Cell.* 2007; 130:1032–1043. [PubMed: 17889648]

- [27]. Wu J, Brown SHJ, von Daake S, Taylor SS. PKA Type II α Holoenzyme Reveals a Combinatorial Strategy for Isoform Diversity. *Science*. 2007; 318:274–279. [PubMed: 17932298]
- [28]. Taylor SS, Ilouz R, Zhang P, Kornev AP. Assembly of allosteric macromolecular switches: lessons from PKA, *Nature reviews. Molecular cell biology*. 2012; 13:646–658. [PubMed: 22992589]
- [29]. Zhang P, Smith-Nguyen EV, Keshwani MM, Deal MS, Kornev AP, Taylor SS. Structure and Allostery of the PKA RII β Tetrameric Holoenzyme. *Science*. 2012; 335:712–716. [PubMed: 22323819]
- [30]. Ilouz R, Bubis J, Wu J, Yim YY, Deal MS, Kornev AP, Ma Y, Blumenthal DK, Taylor SS. Localization and quaternary structure of the PKA RI β holoenzyme. *Proceedings of the National Academy of Sciences*. 2012; 109:12443–12448.
- [31]. Vigil D, Blumenthal DK, Brown S, Taylor SS, Trewella J. Differential effects of substrate on type I and type II PKA holoenzyme dissociation. *Biochemistry*. 2004; 43:5629–5636. [PubMed: 15134437]
- [32]. Vigil D, Blumenthal DK, Heller WT, Brown S, Canaves JM, Taylor SS, Trewella J. Conformational differences among solution structures of the type I α , II α and II β protein kinase A regulatory subunit homodimers: role of the linker regions. *Journal of molecular biology*. 2004; 337:1183–1194. [PubMed: 15046986]
- [33]. Vigil D, Blumenthal DK, Taylor SS, Trewella J. Solution scattering reveals large differences in the global structures of type II protein kinase A isoforms. *Journal of molecular biology*. 2006; 357:880–889. [PubMed: 16460759]

Highlights

1. The crystal structure of PKG (78-355) identified a novel helical subdomain called the switch helix (SW)
2. The SW anchors opposing protomers
3. This arrangement appears to be crucial for enzymatic activity

Figure 1. Domain architecture of PKG1 α from *Bos taurus* showing the placement of the dimerization domain (DD), autoinhibitory fragment (AI), regulatory (A/B), and catalytic domains.

Figure 2.

A multiple sequence alignment colored by BLOSUM62 score of PKG I and II isoforms. a) The conservation of leucine and isoleucine residues for the dimerization domain constructed of a leucine-zipper motif. b) The sequence conservation of the nucleotide binding sites for the high-affinity A-site and the low affinity B-site is shown. The phosphate-binding cassettes - highlighted in red - are highly conserved sites involved in binding of the cyclic nucleotide within the two sites. In type I isoforms, both sites are wholly conserved. There is divergence between type I and II isoforms between the sequence of the two sites, although the B-site is more highly conserved across all isoforms.

Figure 3.

The structure of the regulatory subunit of PKG1 α in the two separate dimer arrangements possible in the C2 space group. a) The *iso* organization of the regulatory dimers showing the antiparallel alignment of nucleotide binding sites from adjacent asymmetric units. b) The SW dimer showing the interaction of the switch helices from opposing protomers with the B-site from opposing protomers within the asymmetric unit.

Figure 4.

A multiple sequence alignment showing the conservation of the knob/nest configuration. a) The sequence of the nest site is conserved throughout PKG isoforms (yellow) except for an isoleucine to lysine mutation along αA (red arrow). b) The knob motif is only conserved in the type I isoforms of PKG and is denoted by an FFANL sequence that was found to sequester both phenylalanines in the structure of PKG1α [17].

Figure 5.

The structure of the cyclic nucleotide binding B-site from the structure of PKG1α showing the nest in the a) *apo* and b) *holo* configuration. The nest is composed of a hydrophobic pocket that is formed by the N3A motif, β6 and the PBC from the nucleotide binding site that sits proximal to the nest, and αB. When the knob is bound, F350 and F352 sit in the hydrophobic pocket and bury over 660 Å² of surface area. The movement of the PBC, and L294, upon binding of cGMP could potentially provide additional surface area to bury F351 and increase the free energy of dissociation of the two monomers.

Figure 6.

A structural alignment of the nucleotide binding B-site from PKG1 α (green, PDBID:3SHR) and the A-site from PKG1 β bound to cAMP (magenta, PDBID: 3OCP) and cGMP (cyan, PDBID: 3OD0) [16, 17]. Movement in the PBC is associated with cyclic nucleotide binding. This degree to which this movement occurs appears to be dictated by the type of nucleotide present. When cAMP is bound, only a shift of 3.3 Å is recorded, compared to a movement of 4.8 Å upon cGMP binding. Moreover, this is the only site where there is a RMSD of more than 1 Å within the nucleotide binding site.

Figure 7.

The structures of the regulatory domains of *holo* PKG1 α (green) and *apo* PKA:RI α (black) with the A-sites for nucleotide binding aligned using a PyMOL all-atom alignment (residues PKG:78-198, PKA:110-230) [17, 26]. a) A side view showing the bowing of the B/C helix associated with PKG. b) a view down the B/C helix from the B-site showing a conformational change in the relative position of the B-site relative to the A-site. This torsion of the A and B-sites in PKG is likely due to the cAMP bound to the A-site which may initiate the release of the catalytic subunit prior to cGMP binding to the B-site, causing complete retraction of both domains to a closed conformation similar to that previously reported for PKA [25].

Figure 8.

A hypothetical model of PKG activation in type I isoforms that contain the SW helix motif. Under inactive conditions, the catalytic domains interact with the regulatory construct and the SW helix in a similar fashion to the interaction of the PKA catalytic domain with αA and its regulatory domain. Under saturating conditions of cGMP, the release of the catalytic domains would promote an interprotomer dimer formed by the interaction of the knobs on the SW helices with their respective nests as seen in the structure of the 78-355 construct.