The First Crystal Structure of cyclic GMP-dependent protein kinase Iβ dimerization domain reveals the molecular features of an extended leucine/isoleucine zipper.

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Short Abstract — Targeting of cGMP-dependent protein kinase (PKG) provides a mechanism for substrate specificity and is mediated by the dimerization/docking (D/D) domain. Although D/D of PKG Iβ has been shown to binds to TFII-I and IRAG, the structural detail was unknown. To understand its targeting mechanism, we solved crystal structures of PKG Iβ D/D. The structure reveals two helices warping around into a left-handed helix forming a novel leucine zipper. Surprisingly, it also shows positive residues at the d positions forming interhelical interactions with negative residues at the e positions. These interactions flank the docking surface and thus may stabilize the surface.

Keywords — cGMP-dependent protein Kinase, Leucine Zipper, Coiled-coil, heptad repeats, Protein-protein interaction, TFII-I, and IRAG

I. Purpose

Cyclic GMP-dependent protein kinase (PKG), which belongs to the AGC family of serine/threonine kinases, is one of the major intercellular receptors for cGMP. Two different genes encode soluble type I (PKG I) and membrane bound type II (PKG II) in mammalian cells. In addition, PKG I exists as two splice variants (α and β) that differ in the first 100 amino-acids with only 36 % identity at this region. Although PKG Iα and β have identical cGMP binding and catalytic domains, they are fundamentally different in cGMP dependence for activation, substrate specificity and tissue distribution[1].

Due to its high degree of sequence similarity to cAMP-dependent protein kinase (PKA), and the lack of structural information, current models for the regulation of PKG are largely based on PKA. Like PKA, PKG contains a dimerization domain, followed by an inhibitory domain and nucleotide binding sites. Like PKA, cGMP binding to the regulatory domain of PKG is thought to cause a major conformational change in the regulatory domain, which then releases and activates the catalytic domain [2]. Unlike PKA, the regulatory and catalytic domains of PKG are contained on a single polypeptide chain, and while dimerization of PKA is mediated by a four-helix bundle [3], PKG dimerization is mediated by a leucine zipper [2]. The N-terminal dimerization domains of both PKA and PKG are known to provide a stable docking surface for interacting proteins that target the kinases to specific cellular locations.

Because PKGs are broad specificity kinases that can phosphorylate a large set of substrates, they need to be targeted close to substrates in order to be specific. Targeting of PKGs is uniquely mediated by a class of proteins called G-kinase anchoring proteins (GKAPs) [4]. GKAPs bind to the N-terminal region of the R-domain that dimerizes and co-localizes the kinases with their substates and downstream effectors. Although the N-terminal dimerization domain of PKG Iβ has previously been shown to provide a unique docking surface for the general transcription factor TFII-I and the inositol 1,4,5-triphosphate receptor associated PKG substrate (IRAG) [5,6], the structural detail of this protein-protein recognition surface was unknown. In order to understand the subcellular targeting mechanism of PKG Iβ, and the molecular detail of the protein docking motif, we solved a crystal structure of the N-terminal domain of PKG Iβ at 1.9 angstrom and 2.1 angstrom.

II. Conclusion

Here, we report the first crystal structure from cGMP dependent protein kinase. Although a small domain of fifty residues, the structure reveals two parallel helices warping around each other into a left-handed helix and forming an extended leucine/isoleucine zipper with 10 pairs of leucine/isoleucines packing in a "knobs-into-holes" manner. Whereas a cluster of four leucines caps the N-terminus, a pair of tyrosine residues caps the C-terminus of the zipper. Most surprisingly, the structure also reveals two positively charged residues at the "d" positions (rather than at the canonical "g" positions) forming interhelical electrostatic interactions with two negatively charged residues at the "e" positions in a mirror symmetry fashion. The four pairs of electrostatic interactions flank a negatively charged surface previously shown to bind TFII-I and IRAG and may aid in stabilizing this important docking surface.

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