The crystal structures of PKG Iβ (92-227) with cGMP and cAMP reveal the molecular details of cyclic-nucleotide binding

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Background
Cyclic GMP is a crucial second messenger that translates extracellular signals into a variety of cellular responses. As a central mediator of the Nitric Oxide-cGMP signaling cascade, which regulates vascular tone, platelet aggregation, nociception and hippocampal/cerebellar learning, Cyclic GMP-dependent protein kinases (PKGs) represents an important drug target for treating hypertensive diseases and erectile dysfunction.

The fidelity of the NO-cGMP signalling pathway is largely dependent on PKG’s ability to selectively bind cGMP over cAMP. Although both cGMP and cAMP bind and activate PKG, cGMP preferentially activates PKG 60-100 fold better than cAMP; yet, little is known about the molecular features required for the cGMP selectivity of PKG. We have investigated the mechanism of cyclic nucleotide binding to PKG by determining crystal structures of the amino-terminal cyclic nucleotide-binding domain (CNBD-A) of human PKG I bound to either cGMP or cAMP.

Results
The crystal structures of CNBD-A with bound cAMP or cGMP reveal that cAMP binds in either syn or anti configurations whereas cGMP binds only in a syn configuration, with a conserved threonine residue anchoring both cyclic phosphate and guanine moieties. The structure of CNBD-A in the absence of bound nucleotide was similar to that of the cyclic nucleotide bound structures.

Surprisingly, isothermal titration calorimetry experiments demonstrated that CNBD-A binds both cGMP and cAMP with a relatively high affinity, showing an approximately two-fold preference for cGMP.

Overall structure of the PKG Iβ CNBD-A:cGMP complex showing on the left, the PKG Iβ CNBD-A:cAMP complex...
complex in the middle, and the partial apo on the right. All three crystals contained more than one molecule per unit cell, which enable us to sample different modes of interaction with cyclic nucleotides. The phosphate binding cassette (PBC) is shown in yellow, the αβ helix in red and N- and C-termini are labeled. For the cGMP and cAMP complexes, bound cyclic nucleotides are shown with the Fo-Fc omit map electron density.

Cyclic nucleotides interact with the cGMP pocket. Both cGMP and cAMP bond in the cGMP binding pocket are shown on the far left and right and their Isothermal titration calorimetry data binding to the PKG Iβ CNBD-A shown in the middle. The cGMP-binding site is marked with three different sites: the short P-helix together with conserved glutamate and arginine residues at the PBC which captures the sugar phosphate (Site 1); a key residue, Thr193 at the end of PBC that bridges the cyclic phosphate to the guanine ring (Site 2); and the β5-strand that provides a unique docking site for the guanine ring (Site 3). Unlike cGMP, cAMP binds in two different configurations, anti in one molecule (shown on the far right panel) and syn in the other with different sets of contacts.

Although the sugar phosphates share the same set of contacts with the protein at site 1, each purine ring of cAMP shows different contacts with the protein at sites 2 and 3. The calorimetric measurements for cAMP and of cGMP binding to PKG Iβ (92-227) were carried out using a VP-ITC calorimeter (MicroCal LLC, Northampton, MA).

Conclusion
Our findings suggest that CNBD-A binds cGMP in the syn conformation through its interaction with Thr193 and an unusual cis-peptide forming residues Leu172 and Cys173. Although these studies provide the first structural insights into cyclic nucleotide binding to PKG, our ITC results show only a two-fold preference for cGMP, indicating that other domains are required for the previously reported cyclic nucleotide selectivity.

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