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Oral presentation

Bacterial GAF domains

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The cyanobacterial adenylyl cyclases (ACs) cyaB1 and cyaB2 contain tandem GAF domains which are located N-terminally. The tandem GAF ensemble confers cAMP regulation on the cyclase catalytic domain similar to the cGMP-stimulated mammalian phosphodiesterases (PDEs). We have solved the structure of the cyaB2 tandem GAF domain. It is an anti-parallel dimer with cAMP bound to all four binding sites. This is in contrast to the structure of the PDE2 tandem GAF domain which is a parallel dimer with cGMP bound only by the GAF-B domains.

In a chimera of the cyaB2 tandem GAF domain with the cyaB1 AC cAMP causes highly cooperative allosteric enzyme activation (>400-fold; EC_{50} = 1 μM). The cyaB2 GAF domains, like the cyclic nucleotide PDE GAF domains, contain conserved $NK(X)_nF(X)_3DE$ motifs that when mutated in the PDEs abrogate cyclic nucleotide binding. Here, we have mutated the aspartates within this motif to determine which of the cyaB2 GAF domains actually is directly involved in signalling. Single or double Asp/Ala mutants in either GAF-A, GAF-B or both cyaB2 GAF domains still show positively cooperative cAMP stimulation. The cyaB2 GAF $NK(X)_nF(X)_3DE$ motifs contain unusual inserts of 14 (GAF-A) and 19 (GAF-B) amino acids which are not present in the tandem GAF ensembles of cyaB1 or mammalian PDEs. Constructs having these inserts deleted including those with a single Asp/Ala mutation in the $NK(X)_nF(X)_3DE$ motif, are activated by cAMP, yet to a lesser extent (<100-fold). However, in a double D/A mutant of the shortened construct stimulation by cAMP iss almost completely lost. Strikingly, in all shortened deletion constructs the positive cooperativity is lost suggesting that the inserts play a role in domain interaction and/or stabilization of cAMP binding pockets. These results strongly suggest that both GAF-A and GAF-B domains contribute to allosteric enzyme regulation in cyaB2. Further, these data in conjunction with the novel antiparallel structure indicate that one role of the lysine:aspartate salt bridge of the invariant $NK(X)_nF(X)_3DE$ motif is to keep the α4 helix and the α4-β5 linker, which close over the cyclic nucleotide, properly oriented thereby stabilizing the binding pocket which enhances activation and cooperativity.