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Dynamic activation of an allosteric regulatory protein.

Tzeng SR, Kalodimos CG
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Evaluations

Evaluated by Qiang Cui | Steven Van Doren | Torleif Härd

The authors have obtained intriguing evidence suggesting that the activation of an allosteric regulatory protein, CAP, is likely driven by changes in protein motion.

Allostery is prevalent in biology and there have been many recent attempts to characterize the underlying mechanism at the atomic level of detail. Building on their recent analysis of CAP using nuclear magnetic resonance (NMR) relaxation techniques, Kalodimos and Tzeng obtained interesting new insights into the activation of CAP (or its S62F mutant) via a combination of NMR and calorimetry measurements. Specifically, they found that although CAP-S62F-cAMP2 adopts the inactive conformation, the complex still has strong DNA binding affinity; moreover, the DNA binding is associated with a large (favorable) conformational entropy originating from enhanced protein motions induced by DNA binding, which is in contrast to the thermodynamics of DNA binding to the WT CAP-cAMP2 complex (enthalpically favorable and entropically unfavorable). The remaining challenge is to understand how a single site mutation can alter the protein motion pattern so dramatically; this awaits additional experimental and computational studies. For two recent studies relating to protein motion and function, see {1,2}.

References: {1} Gardino et al. Cell 2009, 139:1109-18 [PMID:20005804]. {2} Fraser et al. Nature 2009, 462:669-73 [PMID:19956261].

Competing interests: None declared

Cite this evaluation

Evaluated by:
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19 Mar 2010

Rating 6
Recommended

In the context of prior structural change facilitating allosteric binding of DNA by one transcription factor, dynamics changes emerge that mediate the allosteric binding of DNA by a mutant form of the protein. This body of work is an exemplary synthesis of structural results, thermodynamics and dynamics accompanying the association reactions and highlights alternative mechanisms of allostery.

Tzeng and Kalodimos present an intriguing comparison of the biophysical mechanisms of cyclic adenosine monophosphate (cAMP)-induced allosteric binding of DNA by wild-type (WT) catabolite activator protein (CAP) and a mutant that mostly decouples cAMP binding from DNA binding. cAMP shifts nuclear magnetic resonance (NMR) peaks throughout WT CAP as it triggers the DNA binding domain (DBD) to rotate into the active orientation ready to bind DNA. Subsequent binding of DNA localizes NMR peak shifts and increased rigidity mainly to the DBD. Conversely, mutant CAP-S62F binding of cAMP is accompanied by NMR peak shifts across the cAMP-binding domain (CBD) only, whereas subsequent DNA binding shifts NMR peaks, structure, and dynamics throughout both domains. Despite nearly identical DNA affinity of WT and S62F-substituted CAP, the thermodynamic character underlying the associations is starkly different. WT-CAP-cAMP2 affinity for DNA is driven by favorable enthalpy change overcoming substantial entropic cost. In a dramatic reversal, CAP-S62F-cAMP2 affinity for DNA instead pays an enthalpic cost and is driven by large and favorable entropy gain, much of which appears to be conformational entropy gain occurring largely in the region around the cAMP-binding pocket. A caveat on the authors' quantitative estimates of the conformational entropy gain is that the biophysical NMR community generally questions the site-to-site independence and additivity of these estimates. Regarding the estimates as semi-quantitative to qualitative is prudent. Based on NMR chemical shift trends and fitted NMR relaxation dispersion of millisecond-scale equilibria of the DBD, cAMP-saturated CAP-S62F seems to populate an inactive orientation of the DBD to 98% and an excited state to 2% in which the DBD is properly oriented for binding DNA. While cAMP binding converts WT-CAP to the active structure of the DBD, the conversion of the DBD of CAP-C62F to active binding of DNA apparently waits for the DNA to shift the structure, NMR spectra, and equilibrium population to the active form.

Evaluated by:
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12 Feb 2010

Rating 6
Recommended

Competing interests: None declared

Cite this evaluation

This paper shows that allosteric activation can be achieved by changes in the internal dynamics of a protein without changing the conformation of the structurally inactive state. The results constitute an amendment to the prevailing textbook explanations of allosteric regulation.

Evaluated by:
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Structural Biology
14 Jan 2010

Rating 6
Recommended

The term allostery is derived from the Greek allos (other) and stereos (space). It was coined to define a process by which a molecule (effector) binds to a site on an inactive enzyme that is distant from the substrate-binding site to change the enzyme so that it becomes active. Allostery has, since pioneering work in the mid-60s {1,2}, been established as an omnipresent biochemical mechanism for the regulation of many types of biological functions. The standard view of allosteric regulation is that the effector changes the conformation (structure) of a biomolecule so that it becomes active. A commonly used example is binding of cyclic AMP (cAMP) to catabolic activator protein (CAP) so that the cAMP/CAP complex can bind to DNA to activate transcription. Unliganded CAP only binds DNA weakly, but cAMP binding changes the conformation of the two (distant) DNA-binding domains of CAP so that they can fit into the DNA major groove, resulting in strong binding. In the present paper, however, nuclear magnetic resonance (NMR) experiments show that cAMP acts as an allosteric regulator of a CAP mutant (CAP-S62F) without altering the conformation prior to DNA binding. Yet the cAMP/CAP-S62F/DNA complex is identical to the wild-type cAMP/CAP/DNA complex. How can this be? The authors use NMR relaxation experiments to show that cAMP binding to CAP-S62F results in slow protein dynamics by which the DNA-binding conformation becomes transiently accessible (though only populated in 2% of the proteins at any time). They further show that while DNA binding by wild-type cAMP/CAP results in a reduction of protein dynamics -- a "stiffening" effect -- this is not the case with cAMP/CAP-S62F in which the protein instead becomes more flexible after binding to DNA. This is indeed favorable for binding as it constitutes a gain of conformational entropy and this is why the cAMP-bound CAP-S62F mutant can bind DNA as well as the wild-type complex without initially being locked into its DNA-binding conformation. The effect may be a special case of allosteric regulation, but it may also be common, since dynamics is frequently not considered when discussing allostery and also is not easily measured.

References: {1} Monod et al. J Mol Biol 1965, 12:88-118 [PMID:14343300]. {2} Koshland et al. Biochemistry 1966, 5:365-85 [PMID:5938952].

Competing interests: None declared

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