Allostery modulates the beat rate of a cardiac pacemaker

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The S672R mutation in heart cell ion channels leads to low heart rates and arrhythmia by an unknown route. A multifaceted NMR analysis now demonstrates that this mutant impacts allosteric coupling in domains inside of the cell to change channel activation, providing a mechanistic explanation for phenotypic outcomes.

The continuous and consistent beating of a heart is an amazing thing. Cells in the wall of the heart, in the sinoatrial node, spontaneously produce the electrical impulse that keeps the heart muscle moving. This current, in turn, depends on specialized hyperpolarization-activated cyclic nucleotide-gated (HCN)2 ion channels that create ion influxes, making cells easier to activate (1). The importance of the HCN channels to this process is reflected by the discovery that the S672R mutation in the cAMP-binding domain (CBD; also known as the cyclic nucleotide-binding domain of HCN4, the most abundant channel isoform), is a direct cause of familial lower heart rate or arrhythmia disorder (2). Phenotypically, the mutation causes a constitutive shift toward the auto-inhibitory inactive conformation of S672R, which reduces the required voltage (6). Tetramerization can occur only when cAMP is bound, as this event induces a conformational change (Fig. 1A) in the other intracellular domain, the CBD, that would otherwise clash with the tetrmeric C-linker conformation; in the apo form, then, auto-inhibition is observed (6). Surprisingly, the crystal structure of cAMP bound to an hHCN4 C-linker/CBD construct carrying the S672R mutation, determined by Xu et al. (7), revealed no substantial global conformational changes as compared with the wild-type structure except for a disorder loop on the cAMP-entry path. Together with the experimentally observed 10- and 3-fold decreases of cAMP-binding affinity via isothermal titration calorimetry and fluorescence anisotropy methods, respectively, Xu et al. (7) concluded that the S672R mutation simply weakened the interaction between cAMP and the channel, destabilizing the bound cAMP and promoting the closed state.

Boulton et al. (4) suspected that there may be more to the structural story than the static X-ray images conveyed. The authors used a suite of NMR techniques, including heteronuclear single-quantum coherence spectroscopy (NH-HSQC), a two-dimensional 1H,13C-TROSY, along with subsequent chemical shift projection analysis (CHESPA), to examine the S672R-containing HCN4 (residues 563–724) in the apo and cAMP-bound holo form. Comparing their data to that previously collected for the wild-type protein (6) revealed an extensive perturbation of the dynamics in both apo and holo forms of the S672R mutant. First, they observed a constitutive shift toward the auto-inhibitory inactive conformation of S672R, which, in tandem with a simplified free-energy diagram, explains the negative shift in the activation voltage observed by electrophysiology (Fig. 1B). Second, they fitted koff values for the wild-type and S672R from HSQC analyses that indicated an ~6-fold faster koff value for the mutant, pointing to a S672R-induced acceleration of cAMP release. This corresponding accelerated channel deactivation is consistent with the fact that patients carrying the S672R mutation show a significant drop in heart rate.

Several points make the Boulton et al. paper (4) significant. The manuscript is the first NMR investigation that transforms the dynamic profile of an HCN channel into a simple free-energy landscape for the wild type and the S672R variant in the cardiac pacemaker channels, illustrating clearly how allostery works through modulation of an auto-inhibitory mechanism...
and explaining precisely the corresponding observed phenotype. Of note, based on electron paramagnetic resonance (EPR) and NMR experiments, a recent study (8) indicated that different agonists (cAMP, cGMP, or cCMP) bound to the isolated CBD led to different degrees of conformational changes and extents of stabilization of the active conformation. However, corresponding electrophysiology experiments produced similar increases in the extents of channel activation for the three agonists. This implies that distinct conformational states of the isolated CBD might contribute equally to the release of auto-inhibition for tetramerization of HCN and, in turn, ion channel activation.

In general, this study provides a striking example where NMR spectroscopy can help in explaining observations that cannot be fully explained by static crystal structures (7). Similar approaches can be adopted for other systems where the conformational changes caused by the allostery are not observed in X-ray structure due to crystal packing or other factors (9).

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