Common allosteric mechanisms between ryanodine and inositol-1,4,5-trisphosphate receptors

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yanodine receptors (RyRs) are calcium release channels found in the membrane of the endoplasmic reticulum (ER). We recently described the crystal structure of the RyR1 N-terminal disease hot spot. It is built up by three domains that show clear structural homology with the inositol-1,4,5-trisphosphate (IP3) binding core and suppressor domain of IP3 receptors (IP3Rs). Here we analyze the structural features of the domains in both calcium release channels, and propose a model for the closed state of the IP3R N-terminal region. This model explains the effect of the suppressor domain on the affinity for IP3, and is supported by mutational studies performed previously. We propose a mechanism whereby opening of both RyR and IP3R is allosterically coupled to a displacement of the N-terminal domain from the following two domains. This displacement can be affected by disease mutations, glutathionylation of a highly reactive cysteine residue or ligand binding.

Introduction

RyRs are Ca2+ release channels primarily found in the membrane of the endoplasmic reticulum (ER). Three isoforms have been identified in mammalian organisms (RyR1–RyR3), all of which form ~2.2 MDa homotetrameric complexes. Mutations in RyR1 and RyR2 are known to cause genetic diseases, mostly causing a gain of function leading to unwanted release of Ca2+ into the cytoplasm.1,3 Most cluster in three ‘hot spots’, although the presence of hot spots may be due to a sequencing bias.3 Several high-quality cryoEM studies have described the structures of the RyR in different states with resolutions up to 9.6 Å.4-7 In addition, the crystal structures of the N-terminal domain of RyR1 and RyR2 have been described in references 8 and 9.

We recently solved the crystal structure of the RyR1 N-terminal disease hot spot.8 It folds up in three individual domains (A–C), and docking studies show that it forms a continuum of interactions with the same hot spot in neighboring subunits, creating a ~240 kDa cytoplasmic vestibule.

RyRs show homology with another Ca2+ release channel, the IP3R. Crystallographic studies have described structures of two different fragments in the N-terminal region of this channel. Domains B and C form the IP3 binding core,11 whereas domain A (the “suppressor domain”) reduces the affinity for IP3 by more than 10-fold and was crystallized separately.12,13 How does domain A suppress the affinity for IP3? Undoubtedly, it must be interacting directly with the IP3 binding core, but a crystal structure depicting the IP3RABC architecture has remained elusive.

Results and Discussion

Comparison of the RyR and IP3R N-terminal domains. The N-terminal domains in RyRs and IP3Rs show a large amount of structural homology, corroborated by the amount of sequence identity (19.5–22.2%). In both cases, domains A and B form β-trefoil domains, whereas domain C forms a helical bundle. All three individual domains superpose very well (Fig. 1), but a number of structural differences can readily be observed. Domain A contains two long helices (“arm” domain)
that are replaced by a single short helix in RyR (Fig. 1A), the domain B IP₃-binding loop of IP₃Rs has a completely different conformation (Fig. 1B), and IP₃R domain C has two extra helices (Fig. 1C).

Based on the high structural similarity, we hypothesize that the relative domain arrangement of the N-terminal IP₃R domains in the closed state is very similar to the RyR1ABC structure. We built a homologous model by superposing each individual IP₃R1 domain onto RyR1ABC. Previously, the IP₃R BC domains were crystallized in a compact state that is able to bind IP₃. In our model, they are less compact (Fig. 2A): all helices in domain C rotate around the BC linker towards domain A. This conformational change explains the effect of the suppressor domain (A) on the affinity for IP₃: an energetic penalty is paid to break favorable interactions with domains BC. A detailed examination of the IP₃ binding pocket in IP₃Rs shows that IP₃Rs and RyRs diverge substantially in this region (Fig. 2B).

We are now able to interpret previous information, which supports our IP₃R-ABC model. Analysis of the IP₃R domain BC crystal structures highlighted two conserved surfaces (P-I and P-II), which may be involved in the inter-domain interactions. In our model, the P-II surface (Fig. 3 and green) interacts with domain A. The P-I surface (magenta) does not interact with domain A and is therefore likely to associate with other parts of the protein.

Mutagenesis of domain A identified several residues that affect IP₃ binding. Since domain A suppresses the affinity, mutations that perturb the A-BC interaction should enhance IP₃ binding. The IP₃R1-ABC model supports this hypothesis, as mutations that enhance affinity (Fig. 3 and red) are located at the interface with domain C. Mutations that weaken the affinity (Fig. 3 and blue) locate to other parts of domain A. Another study involving a series of IP₃R N-terminal region chimeras showed that IP₃R1 domain A loops 5 and 7 provide isoform-specific IP₃...
binding properties. In our model, both are involved in the interaction between domain A and C. Thus the non-conserved residues on these loops (Fig. 3 and yellow) may have direct impact on the strength of A-C interactions. Recently two reports highlighted a group of spatially clustered residues (Fig. 3 and orange) in domain A that likely interact with the C-terminal fragment of the IP$_3$R. Our model confirms that all these residues are indeed available to bind other parts of the channel.

Common allosteric movements in opening of RyRs and IP$_3$Rs. A cryoEM study of RyR1 in the open state indicates substantial movements in the region where the hot spot docks, suggesting that the observed domain-domain interactions are not static. In addition, a cysteine in domain A (C36) can be glutathionylated in full-length RyR1, despite being completely buried by the interaction with domain B, suggesting a relative displacement of the two domains. Based on this, we propose the following model for redox modulation of RyRs. Upon channel opening, C36 becomes accessible, allowing redox modification. This destabilizes the domain-domain interactions in the closed state of the channel, reducing the energetic barrier to break these interactions and leading to channel activation. Based on the modeling, we suggest that the same phenomenon occurs in IP$_3$Rs: the relative displacement of the N-terminal domains, and hence channel opening, is facilitated by the binding of IP$_3$ in a pocket formed by domains B and C. Further studies will be needed to determine the exact extent of the domain displacement of both channel types. In this regard, it is unfortunate that the cryoEM map of RyR1 in the open state has not been made available, thus preventing docking experiments with the RyR1 N-terminal domains.

Methods

The crystal structures of the IP$_3$R1 suppressor domain (PDB 1XZZ) and ligand binding core (PDB 1N4K) were superposed onto the crystal structure of RyR1 ABC (PDB 2XOA), using secondary structure matching implemented in COOT. 1N4K was divided into sticks. Magenta: P-1 surface on domain BC (P502, F527, Y557, Q564, Q571, E572, A575, F578 and Q582); Green: P-2 surface on domain BC (E283, V286, K306, Y313, R441, D444, F445, N447, D448 and R506); red: domain A residues (L30K, L32K, V33K, Y34K, F36K and K127); blue: domain A residues (K52K, D97K, V99K and E104K) that, upon mutation, decrease the affinity for IP$_3$; yellow: domain A loop 5 and 7 residues (IP$_3$/IP$_3$: L126/M127, A153/A154, A154/T155, F161/L162) that provide isoform-specific IP$_3$-binding affinity; orange: domain A residues (E20, Y167, K168, L169 and S217) thought to interact with the C-terminal region. This IP$_3$ model supports previous SAXS data.
individual domains (236–433 and 434–602) for two separate superpositions. Figures 1–3 were prepared using PyMOL (Delano Scientific).

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