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Mapping Ryanodine Binding Sites in the Pore Cavity of Ryanodine Receptors

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ABSTRACT Ryanodine (Ryd) irreversibly targets ryanodine receptors (RyRs), a family of intracellular calcium release channels essential for many cellular processes ranging from muscle contraction to learning and memory. Little is known of the atomistic details about how Ryd binds to RyRs. In this study, we used all-atom molecular dynamics simulations with both enhanced and bidirectional sampling to gain direct insights into how Ryd interacts with major residues in RyRs that were experimentally determined to be critical for its binding. We found that the pyrrolic ring of Ryd displays preference for the R^{4892}AGGG-F^{4921} residues in the cavity of RyR1, which explain the effects of the corresponding mutations in RyR2 in experiments. Particularly, the mutant Q4933A (or Q4863A in RyR2) critical for both the gating and Ryd binding not only has significantly less interaction with Ryd than the wild-type, but also yields more space for Ryd and water molecules in the cavity. These results describe clear binding modes of Ryd in the RyR cavity and offer structural mechanisms explaining functional data collected on RyR blockade.

INTRODUCTION

Ryanodine receptors (RyRs) play key roles in a number of fundamental cellular processes at specific junctions between the plasma membrane (or sarcolemma) and the intracellular sarcoplasmic reticulum membrane. The central role of RyRs is to regulate excitation-contraction coupling through the release of calcium ions into the cytosol. RyRs have three isoforms, namely, RyR1, RyR2, and RyR3, expressed in skeletal muscle cells, heart muscle cells, and neurons, respectively. The overall structure of RyRs has a mushroom-like shape composed of four identical multidomain protomers (~4 × 5000 residues) with trans-membrane (TM) and large cytoplasmic regions (1). The cytoplasmic region is involved in control of RyR functions and undergoes significant conformational changes in response to various stimuli (2–4). The TM region of RyRs has a central ion-conducting pore domain connecting the luminal side with the cytosol, sharing many similar features found in other families of ion-selective channels. The pore domain contains a selectivity filter (SF), a pore cavity, and a gate formed by four α-S6 helices. Each TM subunit contains a voltage-sensor-like domain playing a role in conformational dynamics and signal transduction during gating transitions. Dysfunctions of RyRs have been associated with various muscle and neuronal disorders. Aberrant spontaneous diastolic calcium release through RyRs underlies fatal arrhythmias in both heart failure and catecholaminergic polymorphic ventricular tachycardia (2).

To study their functions, ryanodine (Ryd) has been widely used for more than three decades. Depending on its concentrations, Ryd can either stimulate or inhibit RyR-mediated Ca^{2+} release (3,5–7). The binding of Ryd is experimentally demonstrated with at least four possible binding sites with a range of affinities (8). The exact structural mechanisms arising from the different binding affinities and locations of the binding sites remain uncertain. While the binding to the high-affinity site may result in the opening of RyRs, the subsequent binding to the low-affinity sites may induce an opposite effect—a complete closure of RyRs (9). The binding is virtually irreversible because of difficulty in washing out the ligand. The chemical modification of the pyrrolic ring of Ryd by a hydroxyl group renders the process fully reversible (10). It was experimentally shown that the binding of Ryd to the RyR2 is prominently altered by the mutations F4850A, F4851A, L4858A, L4859A, I4866A, and Q4863A (11). Accordingly, these residues were proposed to be involved in the formation of various binding sites. The tentative organization of these binding sites was revealed in the water-pore cavity below the constriction point in the TM domain of RyR1 by cryo-electron microscopy (cryo-EM) (12) at a near-atomic resolution (3.8 Å) (1). So far, this resolution of RyR1 has been the highest

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ever achieved via cryo-EM, comparable to the 3.2 Å-resolution of the first crystal structure solved for a bacterial potassium-selective channel, namely, KcsA (13).

The structural details of Ryd-RyR interactions and tentative binding locations remain poorly described. The impressive resolution of the latest cryo-EM technique (14), however, is insufficient for directly dissecting all binding sites for Ryd in the pore cavity, e.g., by simple geometrical arguments. It is unknown if the closed state of RyR1 can bind a single Ryd in the cavity. To date, experiments indirectly imply that at certain concentrations Ryd does not lock RyRs in the open state, and that Ryd-bound RyR channels can be closed, suggesting that Ryd is probably present in the binding sites in a closed state of RyR2 (15). The experimental challenge in mapping binding sites for Ryd in the pore cavity of RyRs could be solved by using a combination of all-atom molecular dynamics (MD) simulations with a docking approach. The conformational sampling of side-chain relaxation with all-atom MD simulations can improve the quality of the structural models for RyR channels and demonstrate how Ryd could bind in the cavity under thermal fluctuations along different thermodynamics pathways. MD simulations also allow estimation of relative binding free energies to characterize the binding thermodynamically.

Accordingly, this study is aimed to delineate complex molecular underpinnings of Ryd-RyR interactions combining various methods from structural modeling and refinement and various docking approaches (16,17) complemented with enhanced sampling methods used in classical MD simulations (see Materials and Methods) to shed insights into both high- and low-affinity sites in the pore cavity (18,19). Because a reliable force field for such a relatively large ligand like Ryd was missing, we performed step-by-step parameterization of Ryd using previously developed protocols (20–27) and adapted the general automated atomic model parameterization (GAAMP) approach (28) combining quantum mechanical (QM) computations with torsional profile fitting. This parameter set enables future studies of the effects of Ryd on the opening and closure of RyRs. We computed the free-energy profile for the Ryd binding and provided some clues for explaining the experimental findings that mutations F4851A and Q4863A in RyR2 (and F4921A and Q4933A in RyR1) significantly alter the binding affinities of Ryd.

MATERIALS AND METHODS

Docking protocols

We have equilibrated and relaxed a reduced receptor structure before all docking simulations. The reduced RyR1 system includes TM and central domains for all four subunits (see Fig. S1). The central domain includes subdomains (residues from 407 to 4131) responsible for calcium binding, the cytoplasmic subdomain in the voltage-sensor-like domain, and the small C-terminal subdomain known to be essential for the integrity and functional dynamics of the TM section of RyR1. The root-mean-square displacement for the entire system embedded into lipid bilayers and solvated (Fig. S1) reached a plateau $\geq$20 ns. After systematic experimental mapping of binding region by Chen and colleagues (11), we centered the binding sites at the cavity. It is well established that Ryd binding is associated with calcium release blockade. It is also important to emphasize that the structure of the pore domain is the best-resolved element in the large assembly from cryo-EM studies. The Glide-XP (16,29) combined with Induced-Fit Docking (17,30) from Schrödinger Inc. (31) was used to map potential binding poses. We used a grid with bin size of 1.0 Å for both Glide-XP and Induced-Fit Docking. To further explore the viability of the Cryo-EM structure in interpretations of functional studies, enhanced sampling techniques were deployed as described below. We selected top 20 poses with binding scores collected in Table 1 and used two RyR1-Ryd complexes with the distinct modes of binding for the refinement with enhanced sampling and MD simulations.

Parameterization of Ryd

The force field for Ryd (Fig. S2) was parameterized with an in-house version of the software GAAMP (28) to overcome certain parameterization tasks, although the GAAMP server is also available for web-based requests. The detailed procedure, which involves 15 steps based on QM computations for fitting partial charges, van der Waals parameters, bond, angle, and torsional parameters, was discussed by Huang and Roux (28). We used the recommended level of theory for all QM computations (HF/6-31G*). The difficult task in Ryd parameterization was related to fitting torsional surfaces for the molecule with >10 torsional angles needing parameterization. To efficiently train initial parameters, we opted to use CGENFF-based (20–27) torsional parameters to approximate energy profiles that bear similarities, with individual QM torsional scans. This approximation leaves only six dihedral angles for parameterization to have approximated energy profiles. All six torsional surfaces were scanned and fitted to reproduce the QM energies with an uncertainty of $\leq$4 kcal/mol (Fig. S3). The Protein Data Bank (PDB) and protein structure files of Ryd with topology and force field parameters are collected in the Supporting Material.

MD simulations

The MD simulations reported in this study included only the TM domain composed of helices S3–S6 (residues 4787–4945) to focus on the binding of Ryd in the pore cavity. The TM domain of RyR1 was then embedded into the preequilibrated DMPC lipid bilayer solvated with 0.15 M NaCl aqueous solution (Fig. 1 visualized by the software VMD (32)). The CHARMM36 force fields for protein, lipid, water, and counterions were used for all simulations. It is important to note that recent data on divalent cation interactions with proteins show significant issues with nonpolarizable force-field parameters for Ca^{2+} (33) and therefore no Ca^{2+} was

TABLE 1 Docking Free Energies for the First 20 Poses

Pose	ΔG (kcal/mol)	Pose	ΔG (kcal/mol)
1	−12.97	11	−9.00
2	−12.65	12	−8.97
3	−10.70	13	−8.85
4	−10.15	14	−8.57
5	−9.86	15	−8.48
6	−9.86	16	−8.35
7	−9.84	17	−7.51
8	−9.74	18	−6.78
9	−9.73	19	−6.71
10	−9.39	20	−6.46