The Location of Binding Sites for 2-GBI in the Voltage-Gated Proton Channel

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Abstract

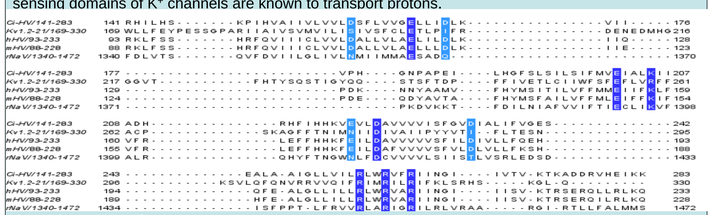
Voltage-gated proton channels (Hv1) play important roles in the respiratory burst, in pH regulation, in spermatozoa, in apoptosis, and in cancer metastasis. The ability to block them selectively is an important target for drug development and for biophysical studies of channel function. Recently, 2-guanidobenzimidazole (2-GBI), a selective and state-dependent blocker of Hv1 channels was identified. However, the exact location of the binding site for a blocker as well as its apparent state-dependence has yet to be established. Recently, we have investigated (Chamberlin *et al*[1]) the structure of the closed and open states of the voltage-gated proton channel through a combination of modeling and experimental analysis. To understand the mode binding and blocking and to hopefully identify the binding pocket, an initial docking study was performed for all of the ligands reported by Hong *et al*[2] as having significant binding affinity with the open channel monomer.

We tested whether our open- and closed-state models could explain the state-dependence of the binding of the guanidine analogue 2-guanidobenzimidazole (2-GBI) from the cytosolic side of the channel. A combination of free-energy simulations and molecular docking established the architecture of the cytosolic binding site for 2-GBI. The site is centered near F198, consistent with the experimental data allowing for interactions between the positively-charged guanidine moiety of 2-GBI and the negatively-charged residues E201, D222, and E219. The aromatic ring of 2-GBI is stabilized by residues in the proximity of F198. In the closed state model, E201, D222, and E219, are occupied by the guanidine moieties of R255 and R258 from S4, thereby preventing 2-GBI access to its binding site. Additional binding modes were investigated by a combination of MD and Free Energy simulations.

Our Prior Work (Chamberlin *et al.*[1])

Homology Models

There are no known crystal structures for either the open or closed states. However the crystal structures of the voltage sensing domains of the related K^+ and Na^+ channels are known. As a consequence both states must be modeled using homology models. Musset *et al*[3] did a detailed phylogenetic analysis of the proton channel family and found that proton channels ancestrally are more closely related to the voltage sensing domains of sodium channels. However models based on the voltage sensing domain of the potassium channel appear more stable. Most notably, mutant voltage sensing domains of K^+ channels are known to transport protons.



Open State Homology

The open state was modeled using the Shaker Kv1.2 channel (2R9R)[4] as the template with the sequence alignments of Musset *et al.*[3] The homology template was fitted using Rosetta[4] and the fragments were determined using PSIPRED.

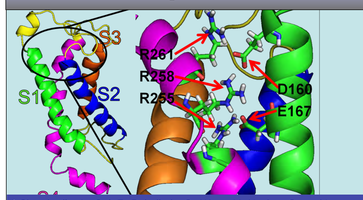
Closed State Homology

The closed state was modeled using the structure of K^+ channel from Pathak *et al*[5] as the template with the sequence alignments of Musset *et al.*[3] The homology template was fitted using Rosetta[6] and the fragments were determined using PSIPRED.

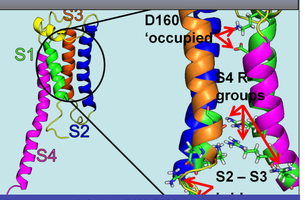
Equilibration and Run

The structures were equilibrated in a DMPC/TIP3 membrane built using the membrane building tools of Im *et al*.[7] Each system was equilibrated at 303.15 K using an NPT ensemble. This was followed with simulation runs of 20 ns. These were performed using NAMD.[8] Subsequent analysis of the system was performed using CHARMM.[9]

Open



Closed



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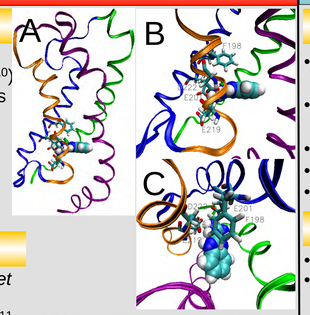
Background

Blocker Studies

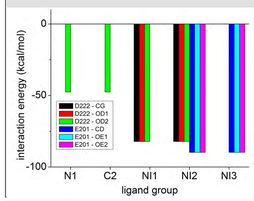
- Guanidine is known to target the voltage sensing domainsin the state-dependent blockade of a variety voltage gated compounds (Sokolov *et al.*[10])
- Using that reasoning Hong *et al.*[2] tested guanidine and several compounds containing guanidine for state-dependent blockage of *Ci*-Hv1 .
- They found that 2-guanidinobenzimidazole (2-GBI) was one of the most effective blockers of the proton channel.
- The found the blockade occured only in the open state when 2-GBI was applied from the intracellular side
- They believe the blocked to be located near F198.

Our Binding Site Studies

- During our work on the gating behavior of *Ci*-Hv1, reported in Chamberlin *et al.*[1], we investigated the binding site for 2-GBI.
- Initial binding poses for 2-GBI in *Ci*-Hv1 were investigated using AutoDock[11] and GOLD[12]
- Top 10 binding poses were investigated using 10 ns equilibration runs with *Ci*-Hv1 in a lipid membrane. (The systems were built using CHarMM-GUI[7])
- The binding energies were computed using the PBEQ module of CHarMM and 100 frames from the equilibration run.

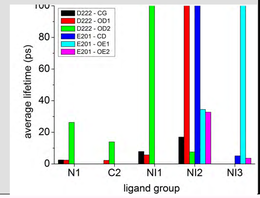


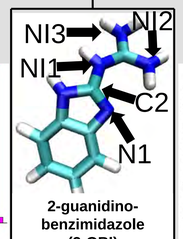
2-GBI bound in open state. (A) The Hv1 channel in the open state with 2-GBI docked inside the pore near F198. **(B-C)** Magmified view of the binding pocked for 2-GBI from the side (B) and bottom (C)



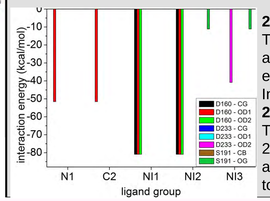
2-GBI – open state interaction energies(left) The decomposition of interactions by residue and by atom s in 2-GBI is shown. Individual entries correspond to atoms in the residue. Interactions energies are measured in kcal/mol

2-GBI – open state contact lifetimes(right) The contact lifetimes decomposed by atoms in 2-GBI and atoms in each residues (D222 and E201) are shown. The cutoff distance is set to be 3.0 Å.



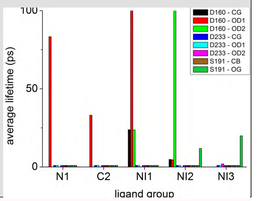


2-guanidino-benzimidazole (2-GBI)



2-GBI – closed state interaction energies(left) The decomposition of interactions by residue and by atom s in 2-GBI is shown. Individual entries correspond to atoms in the residue. Interactions energies are measured in kcal/mol

2-GBI – closed state contact lifetimes (right) The contact lifetimes decomposed by atoms in 2-GBI and atoms in each residues (D160, D233, and S191) are shown. The cutoff distance is set to be 3.0 Å.



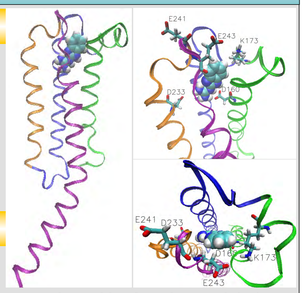
Results

Intracellular Binding Site

- Our docking results confirm the presence of a binding pocket in the open state near F198.
- The favored pose involves E201, E219, and D222 in binding the guanidinium moiety
- Site is occupied in closed state by R255 and R258
- D222 interacts with NI1 and NI2, E201 interacts with NI2 and NI3
- E219 also interacts with the ligand primarily through N1

Extracellular Binding Site

- Extracellular binding site appears to be in the closed state
- The favored posed involves D160 and D233 in binding the gaunidinium moiety
- Site is occupied in open state by R258 and R261
- Binding energies comparable to those of the intracellular binding
- D160 forms long-lived bonds to the guanidinium moiety and to a lesser extent D233, however binding to one excludes the other.



2-GBI bound in closed state. (A) The Hv1 channel in the closed state with 2-GBI docked at the extracellular entrance. **(B-C)** Magmified view of the binding pocked for 2-GBI from the side (B) and top (C)

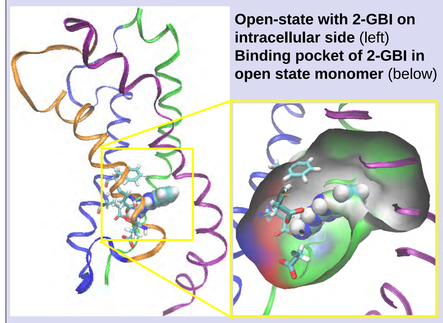
Build a Better Blocker

Objective

Given that the binding site appears to be well defined and that there appears to be potential interactions with other residues neat the binding pocket we sought to create blockers with even higher binding affinities.

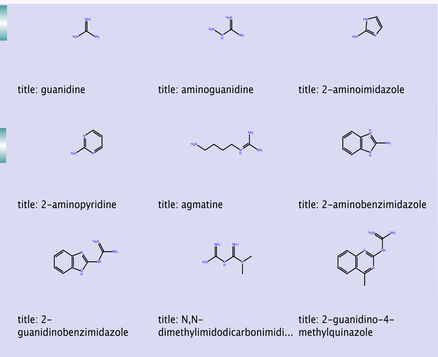
Procedure

1. We used a training set to generate a ligand field model.
2. The ligand field model was used to screen bioisosteres of 2-GBI generated using the Cresset Suite of drug design software.[13]
3. We refined ligands using docking software
4. From the top-scoring docking blockers a smaller subset were chosen for experimental testing.



Open-state with 2-GBI on intracellular side (left)
Binding pocket of 2-GBI in open state monomer (below)

Binding Pocket Colors
Blue: Positive Charge
Green: Hydrophobic
Red: Negative Charge
F198, D222, E201, and E219 shown.



Training set of compounds

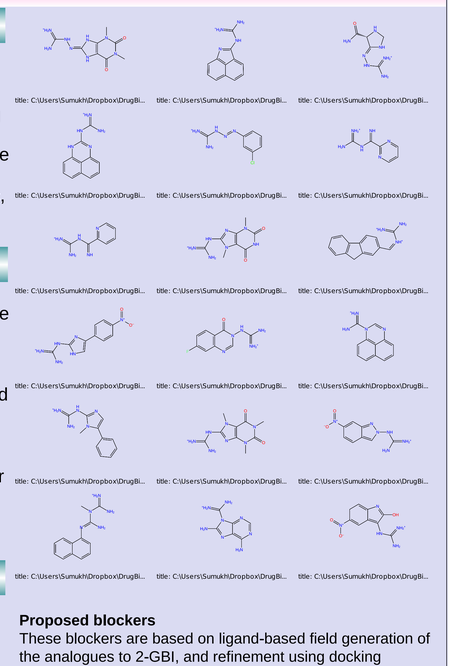
These compounds were taken from Hong *et al.*[1] It was used in the initial ligand-based field generation of the blockers and later used for testing.

Drug Generation

- Our initial approach was to generate drugs with ligand-based drug design using the Cresset Spark module
- We generated a ligand model using a training set of 12 different compounds from the work of Hong *et al.*[1] using the Cresset Forge module.
- We generated bioisosteres of 2-GBI where the guanidine was conserved as was the presence of a ring.
- The identity of the ring included: aromatic, non-aromatic, and heteroatomic variants
- The length of the linker to the gaunidine was varied

Drug Refinement

- The initial set of compounds were ranked according to their similiarity to the ligand field model, among these the top 500 were chosen for further testing.
- The top 500 compounds were tested in both the GOLD and AutoDock docking programs to confirm similar results. While the specific order of the compounds varied between docking programs the top 50 contained the same set of compounds.
- From among the top 50 results from the docking software, 23 representative compounds were chosen for testing.



Proposed blockers

These blockers are based on ligand-based field generation of the analogues to 2-GBI, and refinement using docking techniques,

Ongoing

- Obtaining and testing the proposed blockers or their analogues
- Investigation of off-target blockade by proposed blockers
- Testing the proposed extracellular binding site on *Ci*-Hv1