Modeling the binding of ω-conotoxin MVIIA to an N-type voltage-gated calcium channel

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Research Question

How does ω-conotoxin, MVIIA, binding to the N-type calcium channel compare to other toxins MVIIC, GVIA, MoVIB, and agatoxin IVA?

Introduction

Currently, 1.5 billion people in the world suffer from chronic pain, persistent pain that carries on for longer than 12 weeks despite medication or treatment. Management of chronic pain currently includes use of over-the-counter medicines like non-steroidal anti-inflammatory drugs (NSAIDs) or prescription pain medications like opioids. An alternative was approved for the treatment of severe chronic pain in 2004, Prialt. Prialt, also known as ziconotide, is the brand name for a specific type of toxin (conotoxin) released by marine predatory snails in the family *Conidae*. Once ziconotide is in the body, it acts as a pore blocker of N-Type voltage-dependent calcium channels (Cav2.2). Undergraduate students from Nova Southeastern University built a 3D printed model to explain a molecular story about a particular protein, ziconotide, using the crystal structures described in the literature. The model showcases the various structural details that promote MVIIA specificity toward N-Type voltage gated calcium channels and allow it to be used as an analgesic. Furthermore, our research aims to investigate other toxins as potential analgesics for pain and compare their binding energetics to MVIIA. Other conotoxins have been found to act as pore blockers of the N-Type calcium channel: MVIIC, GVIA, MoVIB, and ω-agatoxin IVA.

Figure 1: marine Predatory snail in the family *Conidae* employing a taser and tether hunting strategy to hunt fish

Figure 2: close-up shot of modified radula used by cone snails to deliver venom

Methods

The protein database file, 7MIX, was used to create this molecular model. The file was imported into *Jmol* and *Pymol*, protein visualization softwares, allowing us to accentuate important features of the protein that were highlighted in the literature. Four other protein database files were investigated: 1CNN, 1TTL, 1OAW, and 6CEG. The program Rosetta Online Server that Includes Everyone (*ROSIE*) was used to perform a local docking search with the other four toxins. The three best scoring toxins were 6CEG, 1TTL, and 1OAW.

Results - Model Information

The model highlights specific features that contribute to MVIIA's binding affinity, restricted to the calcium alpha 1B subunit and the bound conotoxin. Ziconotide contains 25 amino acids, 6 of which are cysteine residues linked in pairs by 3 disulfide bonds. The disulfide bond linkage pattern is a characteristic feature of ω-conotoxins and ensures correct folding of the peptide and stabilization of its structure. In ω-conotoxin MVIIA, the non-cysteine amino acids in the loops determine its binding affinity and calcium channel blocking activity, particularly the second loop located between cysteine-8 and cyseine-15 appears to be the most important in directing selectivity towards the N-Type channels and away from P/Q Type channels (McGivern, 2007). Ziconotide does not directly seal the entrance to the vestibule of the selectivity filter. Instead, it blocks ion entrance by neutralizing the outer electronegativity and sterically hindering the ion access path to the entrance of the selectivity filer. To neutralize the acidic residues, ziconotide engages Arg10 and Tyr13 to bind to Asp664. Four of the eight ziconotide-coordinating residues (Thr643, Asp1345, Lys1372, and Asp1629) in Cav2.2 are not conserved in other Cav channels which explains the subtype specificity of pore blockade by ziconotide. The EEEE motif (Glu314, Glu663, Glu1365, and Glu1655) determines the Ca2+ selectivity (Gao et al., 2021). Also included in the model are the receptor's alpha helices and the calcium ion in the receptor. The N terminus and C terminus of the receptor were marked to orient the model.

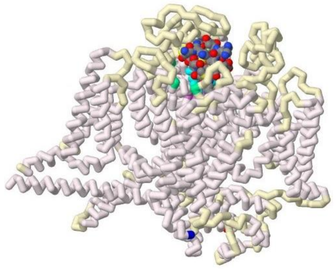

Figure 3: ω-conotoxin, MVIIA, bound to an N-Type voltage-gated calcium channel

Ionic interactions				
Ziconotide (MVIIA)	Arg10		Tyr13	
N-type Ca^{2+} channel	Asp664			
EEEE Motif				
N-type Ca^{2+} channel	Glu314	Glu663	Glu1365	Glu1655
Ziconotide Coordinating Residues				
N-type Ca^{2+} channel	Thr643	Asp1345	Lys1372	Asp1629

General:

- Only subunit α-1B is shown of the N-type Ca2+ channel
- N and C terminus of the receptor are marked in blue and red, respectively
- Alpha helices in the receptor are pink
- The rest of the backbone of the receptor is lemonchiffon
- Calcium ion is green held in place with four spacefill glutamates (EEEE motif) of the receptor colored in orchid
- Ziconotide is colored in cpk spacefill
- An important ionic interaction between ziconotide and the receptor is colored cyan
- Eight ziconotide residues specific to this receptor are highlighted in mediumspringgreen

Discussion

Ziconotide is a proven pain-relief drug derived from a conotoxin (MVIIA) that works by acting as a pore blocker of the N-Type voltage-dependent calcium channels. Other toxins have been proven inhibitors of N-type calcium channels. Four of these toxins (pictured) were put through the protein docking protocol, *ROSIE,* and their binding affinities estimated. From the four additional toxins, it was determined MoVIB, GVIA, and IVA show similar binding sites to MVIIA. The original toxin, MVIIA, was found to dock best. One toxin, agatoxin-IVA, was found to have no relevant binding mode. MVIIC, GVIA, MoVIB had relevant binding modes but bound poorly. It appears there are two main binding pockets that the peptides bind to.

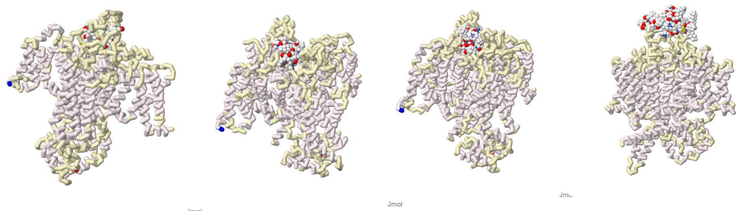

Figure 4: ω-conotoxin, MoVIB, bound to an N-Type voltage-gated calcium channel

Figure 5: ω-conotoxin, GVIA, bound to an N-Type voltage-gated calcium channel

Figure 6: ω-conotoxin, MVIIC, bound to an N-Type voltage-gated calcium channel

Figure 7: ω-agatoxin, IVA, bound to an N-Type voltage-gated calcium channel

Applications

3-D Molecular Models:

- Visually represent molecular processes assisting public health education
- Communicate ideas to others in the scientific community
- Assist in the development of novel drugs that can serve as analgesics

Literature Cited

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