Amino acid substitutions change side chain torsion angles within the pore domains of voltage-gated sodium channels of tetrodotoxin-resistant snakes.

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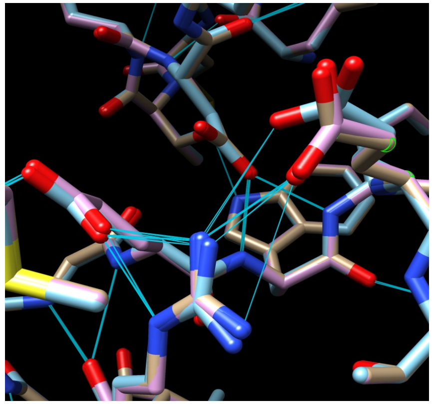
Introduction

Do amino acid changes alter the structure of a protein within the context of its primary structure or through it tertiary structure? Common garter snakes (*Thamnophis sirtalis*) in western North America have evolved different levels tetrodotoxin (TTX) resistance, based on antagonistic interactions with prey. TTX, a neurotoxin, is present in the skin of rough-skinned newt, a prey for some western garter snakes. TTX is the biproduct of an evolutionary arms race between these two species. Local snake populations resistance to TTX is based on several nucleotide substitutions within skeletal muscle voltage-gated sodium channels (VGSCs). The VGSC is a protein that binds to TTX, substitutions exist only in the fourth domain of the VGSCs pore, specifically in the area around the ion selectivity filter. The selectivity filter and outer charged ring of the pore are the areas that interact with TTX. We analyzed and measured amino acid side chain angle changes, investigating the conformational changes within the VGSCs pore region due to amino acid substitution variance. Specifically, whether 1) sequence changes altered the conformational structure of the open VGSC in domain four, 2) if conformational changes cause changes in domains 1-3, associated with mutations in domain four, or 3) if changes in amino acid modify the hydrogen bonds.

Fig 1- Om nom newt (Richard Greene)

Methods

Homology models were rendered by Swiss model, a protein structure homology-modelling server, utilizing a human Nav 1.4 template. We used *T. sirtalis* populations in Benton, Oregon; Willow Creek, California; as well as a reconstruction of the ancestral sequence of all Western Garter snakes. UCSF Chimera was used to visualize, overlay the models, measure torsion angles and the number of hydrogen bonds.



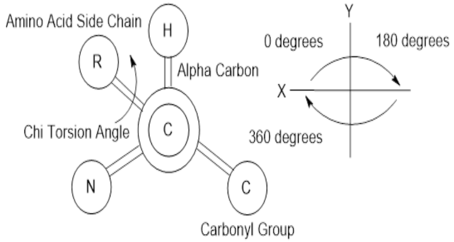


Fig 2- Chi angles are among the 3 possible consecutive angles and 4 atoms that exist in an amino acid side chain, they describe the angular torsion around these bonds.

Results

Mutations within domain four alter the conformation of T. sirtalis VGSCs. Surprisingly, structural changes move beyond local shifts and cause alterations in the bonds and chi angles of domains 1-3. The size of chi angle changes in individual populations track the levels of TTX which is required to inactive 50% of the muscle VGSCs. Beyond the changes in torsion angles, substitutions also shift the hydrogen bonds that interact between domains and with TTX. Resulting in 10 angle shifts greater than 10 degrees, see tables 1 and 2.

Fig 3- Glutamine, 801 contains a shift in the number and placement of the hydrogen bonds that interact between its placement in the outer-negatively charged ring and isoleucine, 794 near the. The reduction in the Willow Creek, CA populations hydrogen bonds suggests a tertiary confirmational shift in the population. While the ancestral and Benton, OR populations remain similar.

T. sirtalis Na_v 1.4 bp	1559------- > -------1571
Ancestral	FEITTSAGWDGLL
Benton, OR	FEVTTSAAWDGLL
Willow Creek, CA	FEVTTSAGWNVLL

Table 3- Amino acid substitutions.

Selectivity Filter Difference						
Domain	Amino Acid	Population	Chi 1	Chi 2	Chi 3	Chi 4
1	Asp 396	Anc	190.478	4.23605		
		Org	0.679	-0.44085		
		Cal	0.102	-3.15524		
2	Glu 798	Anc	294.961	178.751	0.21317	
		Org	0.353	182.207	0.6167	
		Cal	-1.9681	181.644	1.07354	
3	Lys 1273	Anc	277.284	76.9077	177.235	181
		Org	-1.1077	-0.0019	1.144	179.27
		Cal	-2.5987	-1.59	0.642	180

Table 1- The selectivity filter of the VGSC interacts with ions as they pass through the pore of the channel, selectively allowing sodium to travel down its electro-chemical gradient. The filter also interacts with TTX, causing the VGSC to become blocked, initiating the paralysis of the skeletal muscle.

Charged Ring					
Domain	Amino Acid	Population	Chi 1	Chi 2	Chi 3
1	Glu 399	Anc	40.6803	63.005	55.7742
		Org	2.5819	6.3482	-28.8613
		Cal	7.1329	26.2559	-39.7395
2	Glu 801	Anc	193.91	184.56	61.4758
		Org	0.493	-0.022	4.9725
		Cal	0.166	176.017	-42.9361
3	Met 1276	Anc	308.644	305.102	283.994
		Org	-0.1946	-0.9057	-0.6496
		Cal	-0.8474	-0.663	-1.1086
4	Asp/ Asn 1568	Anc Asp	53.3442	304.176	0
		Org Asp	-0.1784	2.875	0
		Cal Asn	11.2437	-6.4464	0

Table 2- The outer negatively-charged ring of the VGSC interacts with TTX as it binds to the channel. The ring's amino acid side chains cause these interactions. Amino acid substitutions suggest a structural change in the confirmation within the pore, affecting TTX binding.

Conclusions

TTX resistance in garter snakes evolved through amino acid substitutions in VGSC sequences of domain four, suggesting an altered protein conformation. Structural changes exist beyond domain four, repositioning key amino acids in domains 1-3's charged ring and selectivity filter, changing how TTX interacts within the pore region. Modification of amino acids led to changes in chi angles and hydrogen bonds, tracking binding affinity of muscle VGSCs.

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