ANALYSIS OF PARALYSIS: Modeling the Binding Site of α-bungarotoxin to Nicotinic Acetylcholine Receptors

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I. Abstract

Myasthenia gravis, a disease characterized by muscle fatigue and weakness, affects three to thirty million people a year. The venom of certain snakes generates a similar response in its prey, causing paralysis. In both cases, symptoms result from interference with neuromuscular transmission. In normal neuromuscular function, binding with acetylcholine creates changes in nAChRs to catalyze ion-selective transmembrane pore openings. Ions of electrical signals from the nervous system enter the muscle cell through a neuromuscular junction, inducing movement. The binding of the snake toxin α-Btx to nAChRs thwarts the electrical signal in rodents. The Whitefish Bay High School SMART (Students Modeling A Research Topic) Team used 3D print technology to model the site where α-bungarotoxin (α-Btx) binds to nicotinic acetylcholine receptors (nAChRs). These nAChRs can be located in a neuron's plasma membrane and on the presynaptic and postsynaptic sides of the neuromuscular junction. This binding primarily takes place between the tips of fingers I and II to form a mobile region that is essential for proper binding and the C-terminal loop of toxin, and loop A, loop B, loop C, and the carbohydrate chain in the receptor. The residues Tyr93, Tyr190, Tyr198, and Trp149 of the α1 subunit in α-Btx are inserted into the aromatic cage of the receptor by Arg36 and Phe32 in finger II of α-Btx, blocking the agonists' access to the activation site. Thus, α-Btx prevents the opening of ion channels that allow the passage of electrical signals. α-Btx has become useful in diagnosing Myasthenia Gravis, as the disease antibodies and the snake toxin occlude the same portion of the nAchR. Therefore, these ion channels and nAChRs are key pharmaceutical targets as more in-depth study could lead to medical breakthroughs about diseases including Parkinson's, Alzheimer's, myasthenia gravis, and epilepsy. α-Btx has become useful in diagnosing Myasthenia Gravis, as the disease antibodies and the snake toxin occlude the same portion of the nAchR.

II. Myasthenia Gravis

In the United States alone, there are twenty to two hundred thousand cases of myasthenia gravis, which, when translated from Latin and Greek, literally means "grave muscle weakness." As its name indicates, this disease results in a chronic muscular fatigue caused by a breakdown of the communication between nerve and voluntary muscle cells at the neuromuscular junction, a place where the nerve cells connect with their respective muscle cells. Normal neuromuscular function allows us to make even the smallest movements. However, this neuromuscular function can be thwarted in various ways. One such way, for example, is through the binding of α-bungarotoxin. α-bungarotoxin is a neurotoxin that directly comes from the venom of snakes and binds to the skeletal muscle acetylcholine receptors, causing failure of the neuromuscular transmission, similar to what happens in myasthenia gravis. Researchers have hypothesized that other impediments to normal neuromuscular function can cause other diseases today, including Parkinson's, Alzheimer's, and epilepsy, and when taken too far, death. Therefore, understanding the process of this binding and finding its links to physiological processes can help develop future medications and treatments to prevent paralysis once and for all.

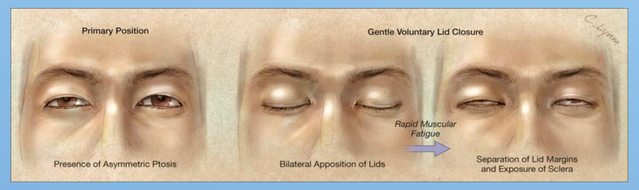


Figure 1. Symptoms of Myasthenia Gravis.

III. Computational Modeling Methods

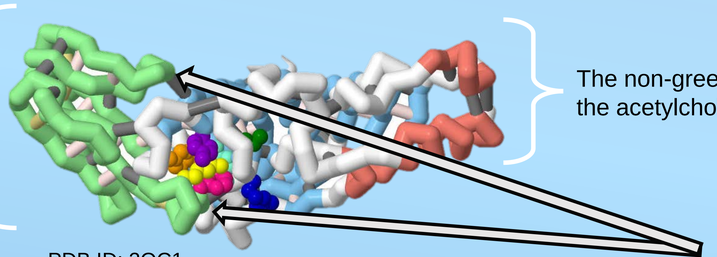
The Whitefish Bay SMART Team uses a computer modeling system called Jmol to visualize our molecule and its respective biological process. Jmol presents a 3-D visualization of the molecule. The 3-D model is then printed at MSOE as a physical representation of the molecule. Having a physical model of the process allows researchers to better understand the details of the binding between α-bungarotoxin and acetylcholine receptors.

IV. α-bungarotoxin in Neuromuscular Junctions

- In normal neuromuscular function, electric signals from the nervous system bind to acetylcholine receptors in the neuromuscular junction. In the case of α-bungarotoxin, it binds to the acetylcholine receptors, preventing electrical signals from binding and transmitting the impulse for muscle movement. When α-bungarotoxin binds to the acetylcholine receptor, it acts like a claw and forms an aromatic cage bond engaging key residues in the active site of this binding. The α-bungarotoxin has fingers that jam into the acetylcholine receptor to form a tight bond. Additionally, the acetylcholine receptor has a carbohydrate chain (not shown in model) that essentially cradles the back of the α-bungarotoxin, aiding in the tight bonding of these two molecules.
- Specifically, the residues tyrosine 93 of finger I of the α-bungarotoxin and phenylalanine 32 of finger II of the α-bungarotoxin have a very strong π-stacking interaction that prevents agonists from binding to the acetylcholine receptor. Agonists are chemicals that bind to and cause a receptor to produce a biological response. In this case, the agonist is acetylcholine.
- Because of its intimate connection with neuromuscular function, the binding of α-bungarotoxin and acetylcholine receptors can be used for research in neuromuscular function and other fields as well.
- Our mentor in particular is using this binding interaction in his studies of zebra fish development.
- In proper zebra fish development, there should be certain numbers of neuromuscular junctions at certain ages, which are mentor detects using α-bungarotoxin and tracking its activity with in neuromuscular junctions. Our mentor hypothesizes that a byproduct of plastic may be hindering the growth of zebra fish.

V. Interaction of α-bungarotoxin and Acetylcholine Receptor

The green represents the α-bungarotoxin that is bound to the acetylcholine receptor.



The non-green area represents the acetylcholine receptor.

These are the fingers of the α-bungarotoxin.

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VI. Biological Significance

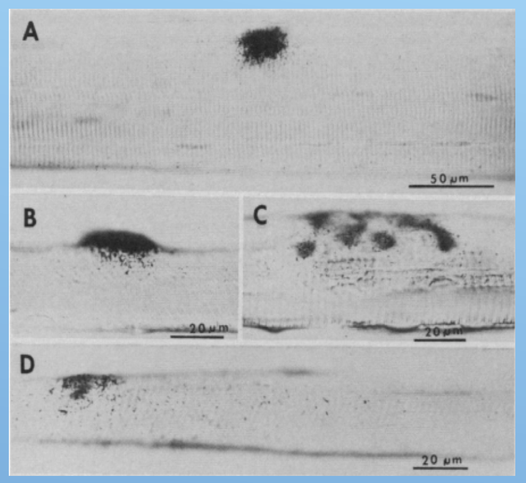

Figure 2. Myasthenia Gravis' affect on neuromuscular junctions.

Patients with Myasthenia Gravis have disorganized neuromuscular junctions where antibodies block the binding of α-bungarotoxin to acetylcholine receptors, causing the binding sites to be defective. In this image, the black represents acetylcholine esterase and the speckles represent α-bungarotoxin. Scientist inserted iodine 125 into the α-bungarotoxin to make it visible. Patients A and B, who do not have myasthenia gravis, show a far greater number of binding sites in a condensed area than patients C and D. In contrast, patients C and D, who have myasthenia gravis have much less binding sites and in a more dispersed area because the defective binding sites in patients C and D do not allow α-bungarotoxin to properly interact with the acetylcholine receptor.

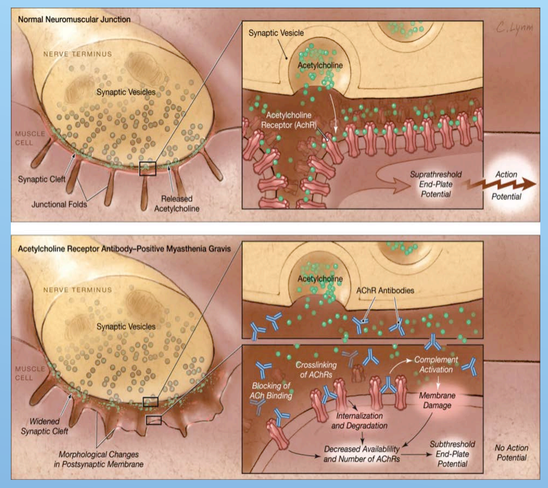

Figure 3. Myasthenia Gravis in the neuromuscular junction.

VII. Bibliography

1 and 3. Images of symptoms of Myasthenia Gravis and Myasthenia Gravis in the neuromuscular junction. (n.d.) Retrieved February 11, 2015, from The Journal of the American Medical Association.

2. D.M. Fambrough, D.B. Drachman, S. Satyamurti (1973). Neuromuscular Junction in Myasthenia Gravis: Decreased Acetylcholine Receptors. *Science.* 182, 4109: 293-295.

PDB ID: 2QC1
Dellisanti, C.D., Yao, Y., Stroud, J.C., Wang, Z., Chen, L., (2007). Crystal structure of the extracellular domain of nAChR alpha1 bound to alpha-bungarotoxin at 1.94 A resolution. *Nat. Neurosc*i.10: 953-962

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