Docking multiple proteins into botulinum toxin type A using computational methods

Brayden Freitas
Mentored by Richard E. Sweeney

Introduction

Botulinum toxin type A (BoNT/A, fig. 1) is the most potent natural toxin known to man. Because of its potency, coupled with the ease of production and ease of usage, this toxin has a strong likelihood as being utilized as a weapon by bioterrorists (Arnon et al., 2001). BoNT/A is specifically an enzyme which cleaves SNAP-25 (Synaptosomal-Associated Protein, 25 kDa) on the carbon backbone using an active zinc ion (Zn^{2+}) in the active site. The SNAP-25 protein complex allows vesicles filled with acetylcholine (ACh) to fuse with the phospholipid bilayer inside of the sending terminal of acetlycholinergic neuronal clefts.

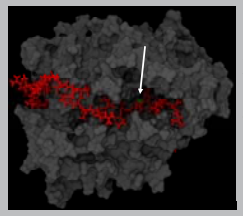


Figure 1: BoNT/A (grey) with SNAP-25 (red) sitting in the active site near the active zinc ion (green, white arrow). SNAP-25 is settled around BoNT/A in a ravine which wraps almost the full way around the protein. A small part of the non-active site end of SNAP-25 can be seen behind the lower right corner of BoNT/A

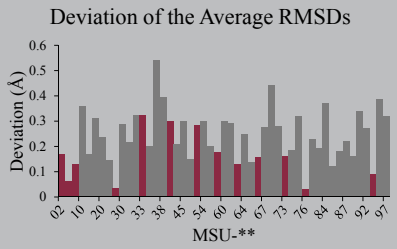
Cleaving the SNAP-25 inhibits the release of ACh, causing a multitude of ailments which manifest themselves between 12 and 72 hours after intoxication. In a wide-spread exposure situation a large amount of cases of flaccid paralysis could occur. The current emergency treatment for flaccid paralysis of the respiratory system is to use a large respirator, of which many hospitals have a limited amount. On top of this, paralysis can last from days to years, requiring long term treatment of multiple people, and potentially leading to a great loss of life. Currently, the most effective inhibitory molecules of BoNT/A are large, expensive to produce, and have low production yields. Because of this, finding small inhibitory molecules is the top priority in many fields of research. Using molecular dynamics simulation programs, small, easy to produce molecules can be computationally docked in to the BoNT/A enzyme, and their viability as inhibitors can be observed.

Methods and Materials

The structures of 50 quinolinol botulinum protease inhibitors were obtained from Dr. Frank Rotella at Montclaire State University (MSU). The structures were digitally drawn ChemDraw (Version 15.0; PerkinElmer, Inc., 2015), and then saved in the *.pdb file format to be imported into Discovery Studio (Release 4.5; Dassault Systèmes: BIOVIA, 2015). The structure of the light chain (LC) of BoNT/A was pre-generated with X-Ray Crystallography and quantitative structure–activity relationship (QSAR) analysis of the crystalline BoNT/A LC (Gul, Smith, & Ahmed, 2000). A script was written for a chemistry at Harvard macromolecular mechanics (CHARMm) simulation program called Discovery Studio, in order to allow the structures of both the protein and ligand to exhibit flexible behavior during docking.

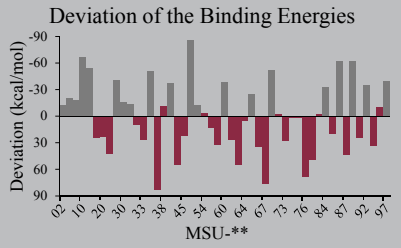
The BoNT/A enzyme is then prepared by having its structural energy minimized, fixing broken loops in its structure, and having all of its residues identified and categorized. In sequence, each molecule and the enzyme are then run through the flexible docking script and the docking position of least energy is determined. A measurement of the binding energy of the inhibitor to BoNT/A was taken and then the system was simulated for 1 ns. The area around the active site had its root mean-squared difference (RMSD) taken after the simulation ended.

Results



Graph 1: The averages of the deviations from the baseline of all 50 RMSDs of the active sites of the BoNT/A and SNAP-25 complexes for each MSU compound are plotted here. The deviations which are significantly different than the baseline are colored grey.

Graph 2: The deviations of the binding energies from the average binding energies of all of the MSU compounds are plotted here. The deviations below the lower 95% confidence interval around the mean are colored grey. The more negative the binding energy, the better the binding.



Of the 50 MSU compounds, MSU-57 had a binding energy which was an outlier. The protein system would not have its energy accurately minimized during the flexible docking step. Nine baseline RMSDs were generated from 1 ns simulations which did not contain any inhibitor.

From the remaining 49 MSU compounds, 12 had both RMSD deviations which were significantly different than the baseline average, as determined by a repeated Welch's *t*-test, and a binding energy deviation which was below the lower 95% confidence interval around the mean are marked.

Conclusion

Twelve small, easily producible molecules have been identified as potentially effective inhibitors of BoNT/A by the amount they change the active site of a BoNT/A and SNAP-25 complex, as well as by their binding energies to the BoNT/A active site. Of these 12 molecules, MSU-36 exhibited an exceptionally large change to the BoNT/A active site (Welch's *t*-test, $\alpha = 0.05$, Graph 1), as well as having a moderately low binding energy (95% confidence interval, Graph 2), and is likely the most promising inhibitor of the set.

Since the SNAP-25 is broken at a specific point in the chain, the active site's shape is important to be consistent (Figure 2). In addition to this, since the active site is very tailored to the shape of SNAP-25, the shape of the whole site area is important to the function of BoNT/A (Figure 2). If the active site is significantly changed, BoNT/A will not be able to function, which is why the RMSD of the site area was measured.

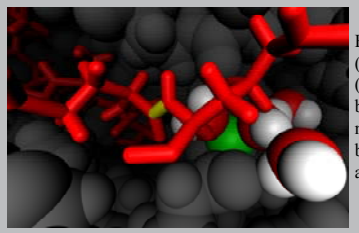


Figure 2: BoNT/A (grey) with SNAP-25 (red) in the active site near the zinc ion (green) and 4 waters (white and red). The bond marked in yellow is the carbon-nitrogen backbone bound which is broken by BoNT/A. Note how compressed the active site is around the zinc.

These observations could be further reinforced with longer run simulations which can be run for longer than 10 ns. This requires access to supercomputers due to the large amount of computational time required.

The observations can move on to the next step of testing by beginning synthesis of the 12 likely candidates and then finding their IC_{50}. Eventually, the inhibitors may be approved for human use.

References

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