Analysis of Alpha Helices in the Receptor Binding Domain in Botulinum Types A, B, D, G

Dakota R. C. Topp[1] and Jeddidiah W. D. Griffin[2]

[1]Milligan University, Milligan, TN 37682

[2]Mars Hill University, Mars Hill, NC 28754

Introduction

Botulinum toxin is a protein produced by *Clostridium Botulinum* and has seven subtypes denoted A-G. Types A and B are both known to cause human botulism, the other five types are non-toxic to humans. The purpose of this study was to analyze the receptor binding domains in type A, B, D, and G in order to correlate toxicity with amino acid sequence and protein structure.

Methods

The four domains of the proteins analyzed were aquired using Protein Data Bank. These sequences were then placed in the programs Chimera, Cystoscope, RING, BLAST, and ChemSketch for analysis purposes. Chimera was used to map the structures and overlay based on amino acid number. RING was used in order to map the network proceeded by Cystoscape to analyze the network for centralization, clustering coefficient, characteristic path length, and average number of neighbors. BLAST protein was used in order to look at percent identity between each of the proteins. ChemSketch allowed for the amino acids of an alpha helix to be drawn and determine differences in singular amino acids in the alpha helix.

Percent Identity

Compounds	Percent Identity
A and B	39.74
A and D	34.63
A and G	40.57
B and D	41.92
B and G	51.94
D and G	38.21

Table 1: Correlation of compounds and their percent identity

The analysis done through BLAST protein looks at the percent similarity of the amino acid sequences between two of the types of botulinum toxin. Type B and G had the highest rate of similarity relative to the other types. However, there was not an overall high rate of amino acid similarity.

Chimera Analysis

Image 1

Image 2 Type A- Blue Type B- Brown Type D- Purple Type G- Green

The Chimera analysis shows a high rate of similarity between all four compounds despite relatively low percent identities. **Image 1** shows an overlay between types A and B and the high rate of structural similarity. **Image 2** is an overlay of all four types demonstrating a high structural similarity. The alpha helical structures are an active component of the binding domain. Despite the high rate of similarity between type B and D there are still major structural differences regarding the alpha helices, particularly the main binding helix consisting of amino acids 856-869.

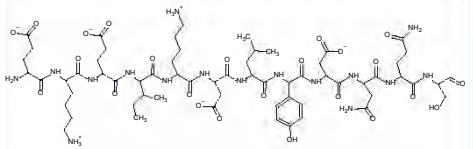
Cystoscope Analysis

Compound	Clustering Coefficient	Network Centralization	Characteristic Path Length	Average Number of Neighbors
A	0.0137	0.017	8.057	3.442
B	0.132	0.018	8.006	3.285
D	0.109	0.019	8.177	3.199
G	0.126	0.019	8.724	3.204

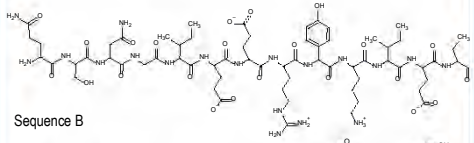
Table 2: Characteristics of each subtype

The Cystoscape analysis shows a high level of characteristic similarity between all four types, consistent with the structural similarity. The clustering coefficient for types A and B were higher than those of D and G, however there was not a significant difference. The network centralization follows a trend of higher toxicity with increased centralization; however, the minimal differences do not indicate a causative agent of toxicity.

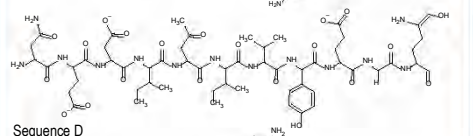
Residue Analysis of α Helix 856-869



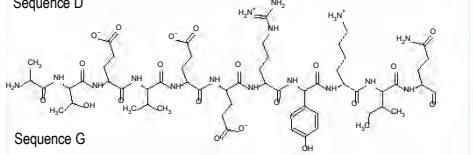
Sequence A



Sequence B



Sequence D



Sequence G

The main alpha helix in the active domain of the receptor binding domain, consisting of amino acid number 856-869 was looked at. The types of amino acids within the toxic and nontoxic types varied in terms of polarity and charge. It was found that in types A and B, shown in the first two amino acid sequences, that there were more polar amino acids within the helix. The presence of arginine, and lysine in a higher frequency leads to the overall positive charge. At bodily pH of 7, type A would have a slight positive charge, and type B would be neutral; in contrast to type D and G having negative charges at pH 7. This difference in polarity and charge could indicate that the differences in the toxicity of types of botulinum toxin is due to these differences. Although all four of these sequences form a similar shaped alpha helix, the charges and polarity associated with each is different.

Conclusions

Through the computational analysis it can be seen there is minimal correlation between many of the factors looked at and toxicity of the specific protein. However, the variance in the alpha helical structure of the active domains of all four types could play a role in the toxicity. As shown in alpha helix 856-859, shown in **Sequences A, B, D, G** there is a difference in the polarity and charge of the main helix at pH 7, this could be indicative of the reasoning for variances in the toxicity between types. There is also a low percent identity between the types, though the structures are very similar. Slight differences of interactions between amino acids, such as charge and polarity, and the interaction binding material could lead to differences in the chemical interaction or the binding ability of the toxins.

Study Limitations

This study looked at only one alpha helix of one domain of the proteins and only looked at four of the seven types.

References

PDB IDs- 5MK6, 6G5K, 3N7J, 3MPP

- Gill DM. Bacterial toxins: a table of lethal amounts. Microbiological reviews. 1982 Mar [accessed 2019 Nov 22]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC373212/?page=2

- Pirazzini M, Rossetto O, Eleopra R, Montecucco C. Botulinum Neurotoxins: Biology, Pharmacology, and Toxicology. Pharmacological reviews. 2017 Apr [accessed 2019 Nov 22]. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5394922/

A special thank you to Dr. Jeddidiah Griffin for overseeing the research.