

Abstract No.9

The Effect of pH on Recombinant C-Terminal Domain of Botulinum Neurotoxin Type E (rBoNT/E-HCC)

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Clostridium botulinum neurotoxins cause botulism syndrome. One of the recombinant vaccines against these neurotoxins is constructed using 93 residues of C-terminal domain of botulinum neurotoxin type E (rBoNT/E-HCC). In this report, we made an effort to investigate the effect of different pH on protein structure to assess if rBoNT/E-HCC could be used as a vaccine for oral administration. Initially, E.coli BL21 (DE3) containing the synthetic gene of rBoNT/E-HCC were cultured and the expressed protein, rBoNT/E-HCC, was purified by affinity chromatography. Structural changes of rBoNT/E-HCC in the presence of different pH (2, 5, 7.4 and 9) were done by various techniques including circular dichroism (CD), fluorescence, aggregation, thermal denaturation and UV-Vis spectroscopy. The aggregation experiments in the presence and absence of DTT indicated that rBoNT/E-HCC at pH 2 is more resistant to thermal aggregation in contrast to higher pH 9. Fluorescence experiments showed the more compact and more stable structure for rBoNT/E-HCC at pH 2, and loosely folded structure at pH 9. Thermal denaturation of protein indicated that the melting temperature was increased with increasing of pH from 2 to 9 up to 2°C. This increment was caused by stabilization effects of increasing amounts of secondary structures which was caused by pH increments. We hypothesize that this finding is not in contrary with aggregation results, because, the nature of forces acting in aggregation and denaturation are not exactly the same. According to our findings we advise further studies of protein from immunological point of view as an oral vaccine.

Keywords: Botulinum Neurotoxin Type E, Ph, Fluorescence, Circular Dichroism, Aggregation, Thermal Studies.

Abstract No.10

Docking of Cladribine and Fingolimod to P53 and RAS exons of DNA

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Some of the newest drugs that are effective on treating Multiple Sclerosis disease (MS) are Fingolimod and Cladribine, but these drugs are carcinogenic. It seems that due to aromatic rings, these drugs are able to bind DNA and can cause cancer by altering DNA structure. Drugs can bind to DNA by covalent and non-covalent bonds, and may cause thermodynamic instability or damage on structure of DNA. These changes can cause abnormal cell division cycle or tumor. Understanding the effect of drugs from the point of structural and mechanical characteristics on the DNA is an important aspect in designing new drugs. Nowadays, docking is essential for designing of new drugs. Fingolimod and Cladribine were docked on four exons of P53 and RAS genes that their sequence mentioned in table 1 by Autodock 4 software. Routine methods and default parameters were used for docking procedure. Results of docking indicate that Van der Waals interactions are greater than electrostatic interaction in connection of these drugs to DNA, in all four sequences. Then these drugs are attached to DNA through hydrophobic interactions. These two drugs were bond to DNA through minor groove. Data indicate that binding of Fingolimod to DNA is stronger than Cladribine, thus it appears that its carcinogenesis effect is greater. Results show Cladribine and Fingolimod can bind to exon one of RAS gene more powerful than other exons.

Keywords: Docking, Cladribine, Fingolimod, DNA.

Abstract No.11

Structural and Allergenicity Properties of Recombinant Wild-type and Cys121Ser Mutant β -lactoglobulin

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