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Proteomics based prediction of antigenicity of iberiotoxin from eastern Indian scorpion

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Abstract- Scorpion venom is an antigenic, water-soluble, heterogenous mixture. The Na^+-channel specific toxins from scorpions are modifiers of the channel gating mechanism. The recombinant DNA vaccines involved targeting multiple antigenic components to direct and empower the immune system to protect the host from infection. Limitation of therapy to the treatment of patients suffering from various adverse reaction and contraindications are always experienced. Antigenic epitope on iberiotoxin - eastern Indian scorpion are important determinant of protection against spider venom. As our knowledge of the immune responses to a protein antigen progressed, it became clear that the whole protein is not necessary for raising the immune response, but small segments (DVDCSVSKECWSVCKDLFG; 4-22) of protein called the antigenic determinant or the epitope is sufficient for eliciting the desired immune response. In analysis predicted antigenic epitope iberiotoxin protein is seen. Antigenic epitope from iberiotoxin is a predicted immunization strategy against various diseases.

Keywords- iberiotoxin, Na+-channel, Antigenic epitope, GOR, Hydrophobicity, Hydrophilicity

Introduction

Scorpion neurotoxin

Scorpion venom is an antigenic, water-soluble, heterogenous mixture. This heterogeneity accounts for the variable patient reactions to the scorpion sting. The various constituents of the venom may act directly or indirectly and individually or synergistically to manifest their effects. In addition, differences in the amino acid sequence of each toxin account for their differences in the function and immunology. Thus, any modifications of the amino acid sequence result in modification of the function and immunology of the toxin. The venom is composed of varying concentrations of iberiotoxin, neurotoxin, cardiotoxin, nephrotoxin, hemolytic toxin, phosphodiesterases, phospholipases, hyaluronidases, glycosaminoglycans, histamine, serotonin, tryptophan, and cytokine releasers. The most potent toxin is the neurotoxin, of which 2 classes exist. Both of these classes are heat-stable, have low molecular weight, and are responsible for causing cell impairment in nerves, muscles, and the heart by altering ion channel permeability. The long-chain polypeptide neurotoxin causes stabilization of voltage-dependent sodium channels in the open position, leading to continuous, prolonged, repetitive firing of the somatic, sympathetic, and parasympathetic neurons. This repetitive firing result in autonomic and neuromuscular overexcitation symptoms and it prevents normal nerve impulse transmissions. Furthermore, it results in release of excessive neurotransmitters such as acetylcholine, epinephrine, norepinephrine, glutamate and aspartate. Meanwhile, the short polypeptide neurotoxin blocks the potassium channels. The binding of these neurotoxins to the host is reversible, but different neurotoxins have different affinities. The stability of the neurotoxin is due to the 4-disulfide bridges that fold the neurotoxin into a structure, thus making it resistant to pH and temperature changes. However, reagents that can break the disulfide bridges can inactivate this toxin by causing it to unfold.

Cardiovascular effect

The Scorpion neurotoxin venom can cause fatal envenoming, but its mechanism of action is unclear. The Na^+-channel specific toxins from scorpions are modifiers of the channel gating mechanism. Venom was tested in vivo in anaesthetized rats and in vitro on isolated cardiac and skeletal muscle preparations. In vivo, the venom caused marked rhythmical fluctuations in blood pressure preceding cardiovascular collapse and death (1). On sheep Purkinje fibres, venom could induce spontaneous action potentials and cause prolongation of action potential duration. In chick biventer cervicis and mouse triangularis sterni preparations, venom enhanced the release of acetylcholine and induced repetitive firing of nerve action potentials in response to single shock stimulation. High concentrations caused stimulation then block of neuromuscular transmission. The main effects of Buthus tamulus venom are likely to be due to toxins that affect the opening of Na+ channels in nerves and muscles. This will cause an increase in the release of neurotransmitters in the peripheral nervous system, which may produce cardiovascular abnormalities and respiratory paralysis.

Scorpion iberiotoxin toxins constitute a family of homologous proteins that exert potent pharmacological effects on potassium or sodium ion channels (2, 3). These proteins are immunogenic and constitute a good model for investigation of the molecular basis of antigenicity. We analyzed the relationships between the structural features of the protein and the location of the antigenic regions: we found that antigenic regions are located at

exposed parts of the molecular surface, i.e. in reverse turns and the alpha helix. These surface parts also correspond to segments of the polypeptide chain, which are most accessible to a large spherical probe modelizing an antibody molecule. Scorpion neurotoxins are a family of homologous, 64 to 65 residue-containing proteins with four invariant disulfide bridges (4). Antigenic epitopes in the *Androctonus australis* neurotoxin and localized them in the amino acid sequence. Deshpande, *et al.* (2005) identified the pulmonary oedema-producing toxin from Indian red scorpion (*Mesobuthus tamulus*) venom (5). Indian red scorpion (*Buthus tamulus*) venom-induced augmentation of cardiac reflexes is mediated through the mechanisms involving kinins in urethane-anaesthetized rats (6). The mechanism underlying the action of Indian red scorpion (*Buthus tamulus*; BT) venom on cardiac reflexes was examined in urethane anaesthetized adult albino rats of either sex. Intravenous injection of phenyldiguanide (PDG) produced reflex hypotension, bradycardia and apnea lasting for > 60s. Augmentation of phenyldiguanide-induced bradycardia by *Buthus tamulus* venom in adult rats (7). In this we can find out the antigenic epitope of neurotoxin M1 iberiotoxin. In analysis predicted antigenic epitope iberiotoxin protein shows successful immunization strategy against various diseases.

Materials and Methods

1. Database searching.

The genomic databases are used to store the vast amount of information issuing from the genome projects. There are many different types of databases available, but for routine protein sequence analysis, primary and secondary, GenBank (8), UniProt (9) databases are initially the most important. We searched and retrieved genome protein sequence of iberiotoxin - eastern Indian scorpion. A sequence is taken directly in FASTA format (10) for ease of use. Sequence was retrieved from Web sites –1.www.ncbi.nlm.nih.gov 2.www.pirgeorgetown.org/

2.Prediction of antigenic peptides

This program predicts those segments from within iberiotoxin - eastern Indian scorpion that are likely to be antigenic by eliciting an antibody response. Antigenic epitope is determined using the method of Kolaskar and Tongaonkar. (11). Predictions are based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. The reported accuracy of method is about 75%.

Method specification –
Program - Prediction antigenic peptides.
Method - Antigen prediction.
Protein sequence – iberiotoxin - eastern Indian scorpion
Format – Raw sequence
Website - http://www.mifoundation.org/

3. Prediction of protein secondary structure

The important concepts in secondary structure prediction are identified as: residue conformational propensities, sequence edge effects, moments of hydrophobicity, position of insertions and deletions in aligned homologous sequence, moments of conservation, auto-correlation, residue ratios, secondary structure feedback effects, and filtering. The GOR method (12) is based on information theory and was developed by Garnier, *et al.* (1996).

Method specification –
Program- Secondary structure prediction
Method- GOR
Protein sequence – iberiotoxin - eastern Indian scorpion
Format– Raw sequence
Website- http://npsa-pbil.ibcp.fr/

4. Finding the location in solvent accessible regions

For setting the solvent accessible regions in protein, type of plot determine the hydrophobic scale and it is utilized for prediction. Sequence of iberiotoxin - eastern Indian scorpion was entered into program- Protein Hydrophobicity plot that characterize its hydrophobic (13) and hydrophilic (14) character, which may be useful in predicting membrane-spanning domains, potential antigenic sites and regions that are likely exposed on the protein surface.

Method specification –
Program- Protein Hydrophobicity Plots
Method- Kyte-Doolittle, Hopp-Woods
Protein sequence – iberiotoxin - eastern Indian scorpion
Format– Raw sequence

Results

Seqeunce of iberiotoxin - eastern Indian scorpion is as:
QFTDVDCSVSKECWSVCKDLFGVDRGKCM GKKCRCYQ
Iberiotoxin - eastern Indian scorpion protein sequence is 37 amino acids residues long.

Prediction of Antigenic peptides

Antibodies find multiple applications in a variety of areas including biotechnology, pharmaceuticals for diagnosis and indeed they are one of the most powerful tools for life science research. These are directed against protein antigens and can recognize either linear or native three-dimensional (3D) epitopes. Antibodies that recognize 3D epitopes require the use of whole native protein as immunogens. Due to various technical reasons this is not always a choice. In such cases, immunization with peptides is the alternative. Antibodies generated in this manner will recognize linear epitopes, and they might or might not recognize the source native protein, but yet they will be useful for standard laboratory applications such as western blots.

Antigenic plot for iberiotoxin - eastern Indian scorpion sequence

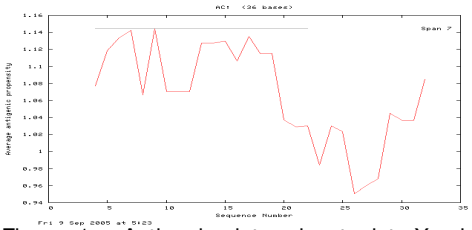

Figure 1 - Antigenic determinant plot. X-axis contains sequence number and y-axis contain average antigenic propensity. There are one (1) antigenic determinant in sequence. The highest propensity pick at start position is 4 to the end position is 22. The sequence is- DVDCSVSKECWSVCKDLFG, having the average propensities for the whole protein is above 1.0725

Table 1- There are one (1) antigenic determinant site in iberiotoxin - eastern Indian scorpion protein sequence:

No.	Start Position	Sequence	End Position
1	4	DVDCSVSKECWSVCKDLFG	22

GOR Alignment

The version IV of GOR has a mean accuracy of **64.4%** for a three state prediction. Above results one matrix corresponds to central amino acid to find in a α.helix, the second for the amino acid being in a β strand, the third a coil, the fourth is a turn.

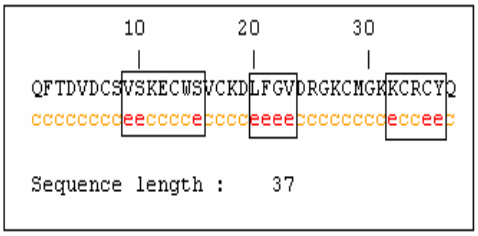

Figure-2- Secondary structure of iberiotoxin protein, which shows Extended strand (Ee) 10 is 27.03%, Random coil (Cc) 27 is 72.97% and no beta turn. Antigenic region of iberiotoxin protein 'DVDCSVSKECWSVCKDLFG'is form a beta sheet region.

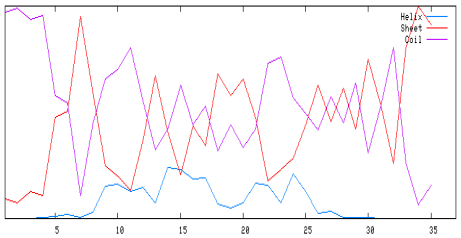

Figure 3- Graphical presentation of secondary structure of iberiotoxin protein.

Solvent accessible regions

Kyte-Doolittle is a widely applied scale for delineating hydrophobic character of a protein. Regions with values above zero (0) are hydrophobic in character. Hopp-Woods scale was designed for predicting potentially antigenic regions of polypeptides. Values greater than zero (0) are hydrophilic and thus likely to be exposed on the surface of a folded protein.

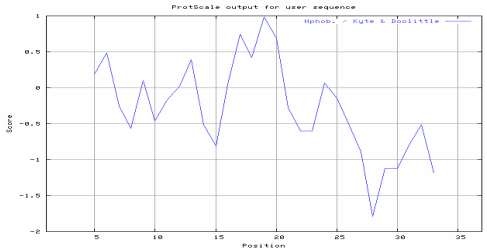

Figure 4- Hydrophobicity plot of iberiotoxin protein. The sequence is- DVDCSVSKECWSVCKDLFG in the plot is above the zero indicating hydrophobic in nature. Input a protein sequence and obtain plots that characterize its hydrophobic character, which may be useful in predicting membrane-spanning domains, potential antigenic sites and regions that are likely exposed on the protein's surface.

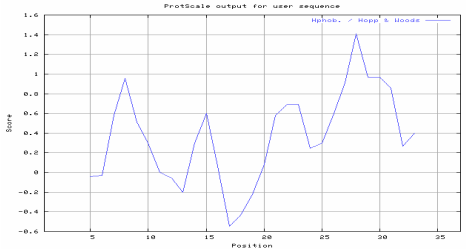

Figure 5 - Hydrophilicity plot iberiotoxin protein. The sequence is- DVDCSVSKECWSVCKDLFG in the plot is below the zero indicates it is hydrophobic in nature.

Discussion

For the prediction of antigenic determinant site of iberiotoxin protein, we got one (1) antigenic determinant site in the sequence. The highest pick is recorded between aa 4 to aa 22. The sequence of AA in this region is 'DVDCSVSKECWSVCKDLFG'. The average propensity for the iberiotoxin protein is found to be above 1.0725 (figure -1). All residues having above 1.0 propensity are always potentially antigenic (Kolaskar and Tongaonkar 1990). Further this region form beta sheet. Thus beta sheet show high antigenic response than helical region of this peptide. A region (4-22) preferably select peptides lying in loops connecting Secondary Structure (SS) motifs, avoiding peptides located in helical regions (Figure – 2 & 3). According to Kyte-Doolittle, Hopp-Woods plot (Figure- 4&5) we can predict that sequence- 'DVDCSVSKECWSVCKDLFG' is hydrophilic in nature. Predicted antigenic epitope is choosing peptides that are in the C-terminal region of the iberiotoxin. Because the N- and C- terminal regions of proteins are usually solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein. These regions

are antigenic in nature and form antibodies. For higher immune response, the peptides could be chemically conjugated to a large carrier protein. However, the process of chemical conjugation is not very reproducible, and uniformity of the peptide density on the carrier protein cannot be ensured. Recent findings show that peptides presented in a particulate form result in enhanced immune responses. One of the major treatments is gene therapy or recombinant DNA vaccines involve targeting multiple antigenic components to direct and empower the immune system to protect the host from chemical reaction. Antigenic epitopes of iberiotoxin are important determinant.

Abbreviations

GOR - J. Garnier, D. Osguthorpe and B. Robson
AA, aa –Amino acid

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