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Comparative *in Silico* Study of Melittin from Honeybee Venom

Neha V. Bhatkar[1], Vishal R. Dhundale[2] and Vijayshree M. Hemke[3]

[1]Department of Zoology, Shri Shivaji College, Akot (MS) 444101 India
[2]NACS College, Ahamadnagar
[3]Jijamata Mahavidyalaya, Buldana (MS)
*Corresponding author: Email: drvmhemke@gmail.com Tel: 09421732680

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ABSTRACT

The bee venom is used for treating a wide variety of conditions from acute tendonitis to chronic back pain to rheumatoid arthritis (RA). The major treatment is gene therapy or 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 epitopes on melittin protein [*Apis cerana*] are important determinants of protection against rheumatoid arthritis. 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 (LVNVALVFYGRVHFLHLCVHFLHLWA, 4-29) of protein called the antigenic determinants or the epitopes are sufficient for eliciting the desired immune response. Immunization cassettes should be capable of immunizing of broad immunity against both humoral and cellular epitope thus giving vaccines the maximum ability to deal with *Apis cerana* immune escape. We have predicted a successful immunization strategy against rheumatoid arthritis.

Keywords: Venom, *In Silico* prediction, Melittin, Epitope, Immunization, *Apis cerana.*

1. INTRODUCTION

The honey bee was a prominent political symbol in the empire of Napoleon Bonaparte, representing the Bonaparty bureaucratic and political system. The life of bees is a mysterious and fascinating one. The bee family, called a colony, functions as an organism; where every bee is like a cell in a body and has its own strictly distinct functions Besides honey, Bee Venom is probably the most well respected aspect of honeybee. It is synthesized in the venom glands of worker as organism which actually changes as the bee mature. It is not the individual bee, but the colony itself that really matters. There are 20,000 known species of bees. The indigenous honeybee species are: *Apis dorsata, Apis cerena, Apis florae*, *Apis mellifera*. The Honeybee produce: Honey, Propolis, Royal Jelly, and Venom.
and queen bees stored in their venom sac. Consequently bees have more potent venom during the summer (2, 5). The darker

colouration is the result of contamination of the venom and oxidation of its Constituents (12). Phospholipase A-2, Adolpin, Apamin, MCDP, Peptide 401, and Melittin are the constituents of bee venom. In this study we focused on melittin.

Melittin is the principal component of bee venom. It is 100 times more potent than hydrocortisone. (6).Melittin and apamin stimulates the human adrenal and pituitary systems to produce cortisol, the natural steroids, one of the body's major anti-inflammatory agents. Naturally produced steroids do not produce the medical complications of synthetic steroids (16). Melittin also stabilizes the lysosome cell membrane to protect against inflammation (8).

Several researchers have examined the anti-inflammatory effects of bee venom on synovial cell-cells lining the joints obtained from human Rheumatoid Arthritis (RA) patients (14, 10,11). Melittin effectively reduced inflammation by inhibiting the critical DNA binding activity of NF-kB (Nuclear Factor kappa B), which directly controls a number of genes involved in immune reactions. The extent of inhibitory effects of melittin in most parameters determined in the present study is similar to or greater than bee venom itself, suggesting that melittin may be a major causative component in pharmacological effects of bee venom (4). Antigenic epitopes on melittin protein (*Apis dorsata, A. cerena, A. florae. A mellifera*) are important determinants of protection against rheumatoid arthritis.

Taxonomy of selected bees

Superkingdom	Eukaryota	Eukaryota	Eukaryota	Eukaryota
Kingdom	Metazoa	Metazoa	Metazoa	Metazoa
Phylum	Arthropoda	Arthropoda	Arthropoda	Arthropoda
Superclass	Hexapoda	Hexapoda	Hexapoda	Hexapoda
Class	Insecta	Insecta	Insecta	Insecta
Superclass	Neoptera	Neoptera	Neoptera	Neoptera
Order	Hymenoptera	Hymenoptera	Hymenoptera	Hymenoptera
Subiorder	Aculeata	Aculeata	Aculeata	Aculeata
Superfamily	Apoidea	Apoidea	Apoidea	Apoidea
Family	Apidae	Apidae	Apidae	Apidae
Subfamily	Apinae	Apinae	Apinae	Apinae
Tribe	Apini	Apini	Apini	Apini
Genus	*Apis*	*Apis*	*Apis*	*Apis*
Species	*dorsata,*	*cerena,*	*mellifera*	*florea,*

2. **MATERIALS AND METHOD**:

Database Searching

Genomic databases are used to store the vast amount of information issuing from the genom projects. There are many different types of databases available, but for routine protein sequence analysis, primary and secondary, Genbank (3), Uniport (1) databases are initially the most important. We searched and retrieved genome protein sequence of melittin protein (*A.dorsata, A.ceren, A. florea, A mellifera*) sequences are downloaded directly in FASTA format (9). For ease of use, sequences were retrieved from web sites are as- www.ncbi.nlm.nih.gov.

Prediction of antigenic peptides

This program predicts those segments from within a melittin protein (*Apis dorsata, A. cerena, A florea. A mellifera*) sequence that

are likely to be antigenic by eliciting an antibody response. Antigenic peptides are determined using method of Kolaskar and Tongaonkar (7). Predictions are based on a table that reflects the occurrence of amino acid residues in experimentally known segmental epitopes. Segments are only if they have a minimum size of residues (http.//www.mifoundation.org)

Method Specification: Program- Prediction antigenic peptides.

Method: Antigenic Prediction.

Protein Sequence: Mellitin Protein (*Apis dorsata, A. cerena, A florea. A mellifera*)

Format: Raw sequence

Website: http.www.bio.dfci.harvard.edu/tools/antigenic.org/.

3. RESULTS AND DISCUSSION

FASTA format of melittin protein (*Apis dorsata, A. cerena, A florae. A mellifera*) is as follows:

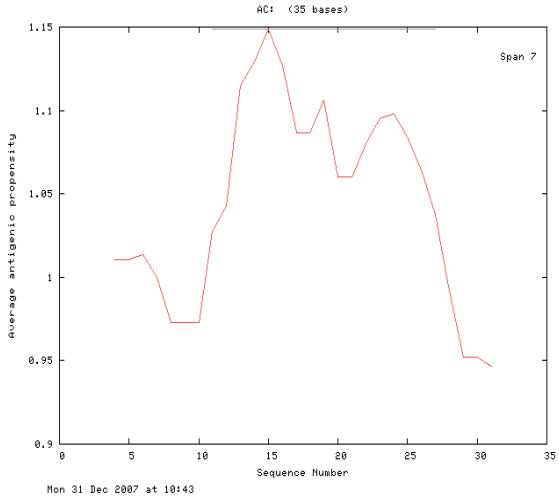
1GIGAILKVLSTGLPALISWIKRKRQE,
2MKFLVNVALVFYGRVHFLHLCVHFLHLWAPEPEPAPEAEAEADAEADPEAGIGAVLKVLTTGLPALISWIKRKRQQG,
3GIGAILKVLATGLPTLISWIKNKRKQ,
4MKFLVNVALVFMVVYISYIYAAPEPEPAPEPEAEADAEADPEAGIGAVLKVLTTGLPALISWIKRKRQQG

Antibodies find multiple applications in a variety of areas including biotechnology, Pharmaceuticals molecular biology for diagnosis and indeed they are one of the most powerful tools for life science research. In analysis of melittin protein, we found two antigenic determinants sites it is highest at start position. The highest peak sequence of antigenic determinants plot indicate antigenic site for the host cell attachments.

Antigenic Specificity is the ability of individual antibody combing site to react with only one antigenic determinant and the ability of a population of antibody molecules to react with only one antigen.

Apis dorsata

Sequence of this protein is 35 residues long. Average antigenic propensity for this protein is 1.0285. Figure: 1 shows the antigenic plot for the sequence.

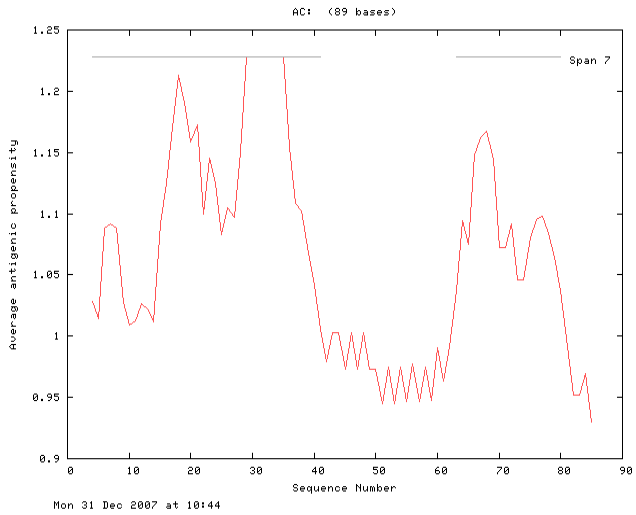


There is 1 antigenic determinants in the sequence:

n	Start Position	Sequence	End Position
1	11	IGAILKVLSTGLPALIS	27

Apis cerena

Sequence of this protein is 89 residues long. Average antigenic propensity for this protein is 1.0582. Figure: 2 show the antigenic plot for the sequence.

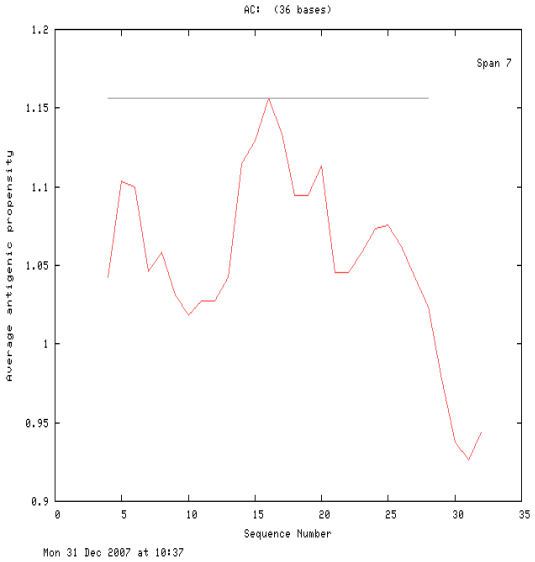


There are 2 antigenic determinants in this sequence:

n	Start Position	Sequence	End Position
1	4	MELAPICEMMKFLVNVA LVFYGRVHFLHLCVHFLH LWA	41
2	63	GIGAVLKVLTTGLPALIS	80

Apis florea

Sequence of this protein is 36 residues long. Average antigenic propensity for this protein is 1.0375. Figure: 3 show the antigenic plot for the sequence.

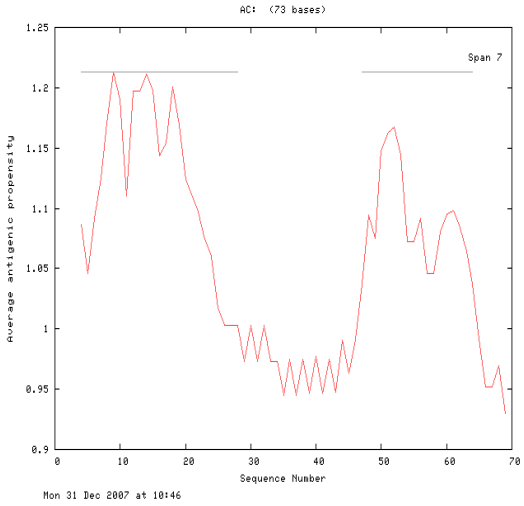


There is 1 antigenic determinant in this sequence:

n	Start Position	Sequence	End Position
1	4	LAPIFLMGIGAILKVLA TGLPTLIS	28

Apis mellifera

Sequence is 73 residues long. Average antigenic propensity for this protein is 1.0480. Figure: 4 show the antigenic plot for the sequence.



There are 2 antigenic determinants in this sequence:

n	Start Position	Sequence	End Position
1	4	MKFLVNVALVFMVVYIS YIYAAPEP	28
2	47	GIGAVLKVLTTGLPALIS	64

For the prediction of antigenic determinants site of melittin Protein of *Apis dorsata*, we got 1 antigenic determinant site in the sequence. The highest peak is recorded seen between amino acid 11 to amino acid 27. The sequence of amino acid in this region is "IGAILKVLSTGLPALIS". The average propensity for the melittin protein is found to be 1.0285.

For the prediction of antigenic determinants site of melittin Protein of *Apis cerena*, we got 2 antigenic determinant sites in the sequence. The highest peak is recorded between amino acid 4 to 41, and 63 to 80. The sequence of amino acid in this region is MELAPICEMMKFLVNVALVFYGRVHFL HLCVHFLHLWA and GIGAVLKVLTTGLPALIS Average antigenic propensity for this protein is 1.0582

For the prediction of antigenic determinant, of melittin protein of *Apis florea* , we got 1 antigenic determinant site in the sequence. The highest peak is recorded seen between amino acid 4 to amino acid 28. The sequence of aa in this region is "LAPIFLMGIGAILKVLATGLPTLIS". The average propensity for the melittin protein is found to be 1.0375.

For the prediction of antigenic determinant, of melittin protein of *Apis mellifera*, we got 2 antigenic determinant site in the sequence. The highest peak is recorded seen between aa 4 to aa 28 and 47 to 64. The sequence of aa in this region is "MKFLVNVALVFMVVYISYIYAAPEP" and "GIGAVLKVLTTGLPALIS". The average propensity for the melittin protein is found to be 1.0480.

All residues having above 1.0 propensity are always potentially antigenic. (http;// ncbi .nlm.nih.gov)

4. CONCLUSIONS

To improve immunogenicity, 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 peptides density on the carrier protein cannot be ensured. Antigenic epitopes of mellitin proteins are important determinants of protection against allergic reactions caused due to sting of honeybee and protection against Rheumatoid Arthritis (RA). The 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 of Protein called the Antigenic Determinants or the epitopes are sufficient for eliciting the desired immune response.

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