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Prediction of 3D Structure of Paralytic Insecticidal Toxin (ITX-1) of *Tegenaria agrestis* (Hobo Spider)

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Abstract

Paralytic insecticidal toxin (ITX-1) (*Tegenaria agrestis*) involved multiple antigenic components to direct and empower the immune system to protect the host from infection. Spider peptide toxins with nanomolar affinities for their receptors are promising pharmacological tools for understanding the physiological role of ion channels and as leads for the development of novel therapeutic agents and strategies for ion channel-related diseases. A 3-dimensional model (3D) was developed for the Paralytic insecticidal toxin of the (ITX-1) of *Tegenaria agrestis* (Hobo spider). A homology modeling method was used for the prediction of the structure. For the modeling, a template protein was obtained by mGenTHERADER, namely the high-resolution X-ray crystallography structure of a FERREDOXIN (1FCA) of Clostridium acidurici. By comparing the template protein a rough model was constructed for the target protein using MODELLER, a program for comparative modelling. The model was validated using protein structure checking tools such as PROCHECK and WHAT IF for reliability. The information thus discussed provides insight to the molecular understanding of Paralytic insecticidal toxin (*Tegenaria agrestis*). The predicted 3-D model may be further used in characterizing the protein in wet laboratory.

Keywords: Structure prediction; ITX-1; *Tegenaria agrestis*

Introduction

The hobo spider, *Tegenaria agrestis*, is a member of the family of spiders known as the *Agelenidae* or *funnel web weavers*. The first record of *Tegenaria agrestis* Walckenaer in the United States was in Seattle,Washington in 1930 [1,2]. European distribution is widespread from Europe to central Asia [3].The current range of *T. agrestis*, originally named the aggressive house spider, includes Washington, Oregon and Idaho [4] as well as Colorado and southern British Columbia [5] Although no medical concerns are associated with *T. agrestis* in Europe [6] conjectures have been made in Washington, Oregon and Idaho, since the late 1980s, due the suspicion that its bite causes necrotic tissue lesions. Approximately 500 species of funnel web weavers occur worldwide; about 300 of these are found in North America, and about 100 species are native to Europe. The hobo spider (*Tegenaria agrestis*) is a member of the genus of spiders known colloquially as funnel web spiders. In the United States, the hobo spider has been considered to be a dangerous species based on a toxicology study on rabbits where lesions appeared after spiders were induced to bite the rabbits, although attempts to replicate the study (by injecting venom to ensure envenomation) have failed to produce necrotic lesions [7,8]. Many peptide toxins from spider venoms share structural features, amino acid composition and consensus sequences that allow them to interact with related classes of cellular receptors. They have become increasingly useful agents for the study of voltage-sensitive and ligand-gated ion channels and the discrimination of their cellular subtypes. Spider peptide toxins have also been recognized as useful agents for their antimicrobial properties and the study of pore formation in cell membranes. Their high insecticidal potency can also make them useful probes for the discovery of novel insecticide targets in the insect nervous system or for the development of genetically engineered microbial pesticides. Insecticidal peptides from Tegenaria agrestis spider venom may have a direct effect on the insect central nervous system. Fractionation of venom from an agelenid spider, Tegenaria agrestis, resulted in the isolation of a family of three peptides with potent insecticidal activity. These peptide toxins, ITX-1, ITX -2, and ITX -3, whose sequences were revealed from cloned cDNAs, each consist of 50-60 amino acid residues, six of which are cysteines [9]. One peptide and ten acylpolyamine toxins (curtatoxins) were purified and identified from venom of *Hololena curta* [10]. Acylpolyamines represent the vast majority of organic components from the spider venom. Acylpolyamine analogues have proven to suppress hippocampal epileptic discharges. Acylpolyamines and peptides from spider venoms represent an interesting source of molecules for the design of novel pharmaceutical drugs [11].

Comparative modeling or homology modeling (HM) is becoming a useful technique in the field of bioinformatics because the knowledge of the three-dimensional structure of a protein would be an invaluable aid to understand the details of a particular protein. However, a solved structure for Paralytic insecticidal toxin (*Tegenaria agrestis*) is not available at the protein data bank (PDB).

Therefore, we created a model of Paralytic insecticidal toxin (*Tegenaria agrestis*) using the X-ray structure of a FERREDOXIN (1FCA) of Clostridium acidurici as template with MODELLER (a comparative modeling program) [12]. The model was validated using protein structure checking tools such as PROCHECK, WHAT IF and ProSA for reliability.

Materials and Methods

Retrieval of target sequence

The amino acid sequence of the Paralytic insecticidal toxin

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(ITX-1) of *Tegenaria agrestis* was obtained from the sequence database of UniProtKB/Swiss-prot (http://www.expasy.org/uniprot) database release 54.0, ID gi|2920713|emb|CAA11839.1 [13]. It was ascertained that the three- dimensional structure of the protein was not available in Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), hence the present exercise of developing the 3D model of the Paralytic insecticidal toxin (ITX-1) of *Tegenaria agrestis* was undertaken. The protein is 68 amino acids in length.

Template searching

An attempt was made to find a suitable template protein for the modeling of the target protein. The template protein was searched through mGenTHREADER, which is an online tool for searching similar sequences, based on sequence and structure-wise similarity [14]. From the homology searching, two templates were selected.Xray crystallography structure of the FERREDOXIN (1FCA) of *Clostridium acidurici* were selected as template proteins.

Sequence alignment

Amino acid sequence alignment of target and template proteins was derived using the Swiss-PdbViewer package (http://www.expasy.ch/spdbv/). Default parameters were applied and the aligned sequences were inspected and adjusted manually to minimize the number of gaps and insertions.

Homology modeling and structure refinement

A rough 3-D model was constructed from the sequence alignment between Paralytic insecticidal toxin (ITX-1) and the template proteins using MODELLER 8v0 (http://salilab.org/modeller/) with parameters of energy minimization value. The model was further checked with WHAT IF and Ramachandran plot at PROCHECK [15,16]. Accessible surface area prediction using VADAR was performed [17]. The rough model constructed was solvated and subjected to constraint energy minimization with a harmonic constraint of 100 kJ/mol/Å^2, applied for all protein atoms, using the steepest descent and conjugate gradient technique to eliminate bad contacts between protein atoms and structural water molecules. Computations were carried out *in vacuo* with the GROMOS96 43B1 parameters set, implementation of Swiss-PdbViewer.

Model validation

The constructed model of Paralytic insecticidal toxin (*Tegenaria agrestis*) was examined for validation using different criteria. In the last step of homology modeling the refined structure of the model was subjected to a series of tests for testing its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK (http://biotech.ebi.ac.uk:8400/cgi-bin/sendquery) analysis. The Swiss-PdbViewer energy minimization test was applied to check for energy criteria in comparison with the potential of mean force derived from a large set of known protein structures. Packing quality of the refined structure was investigated by the calculation of PROCHECK Quality Control value. The Ramachandran plot of phi/psi distribution in the model is developed using PROCHECK for checking non-GLY residues at the disallowed regions. Standard bond lengths and bond angles of the model were determined using WHAT IF.

Results and Discussion

The Paralytic insecticidal toxin (ITX1) protein sequence is 68 residues long as: MKLQLMICLV LLPCFFCEPD EICRARMTHK EFNYKSNVCN GCGDQVAACE AECFRNDVYT ACHEAQKG. By using the bioinformatics tools, a three-dimensional structure model of Paralytic insecticidal toxin of *Tegenaria agrestis*, was constructed by HM. The results are presented here. Many peptide toxins from spider venoms share structural features, amino acid composition and consensus sequences that allow them to interact with related classes of cellular receptors. They have become increasingly useful agents for the study of voltage-sensitive and ligand-gated ion channels and the discrimination of their cellular subtypes. The three-dimensional (3D) structure details of proteins are of major importance in providing insights into their molecular functions. Further analysis of 3D structures will help in the identification of binding sites and may lead to the designing of new drugs.

The protein sequence of the Paralytic insecticidal toxin (ITX1) protein of *Tegenaria agrestis* (Hobo spider) was obtained from the Swissprot sequence database. Multiple alignment of the primary structure of the target protein highlights the degree of sequence

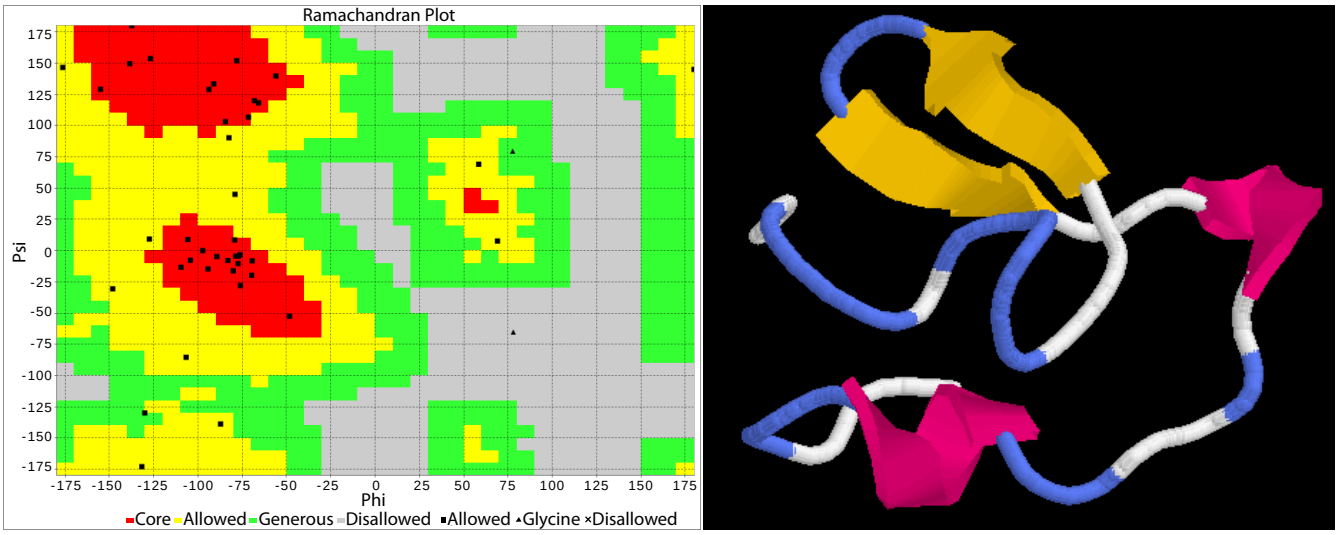

Figure 1: **(A)** Predicted 3-D structure of ITX-1 Ramachandran plot analysis. The Plot statistics are: residues in most favoured regions 32 (78.0%); residues in additional allowed regions 8 (19.5%); residues in generously allowed 1 (2.4%). **(B)** Predicted 3-D structure of ITX-1 of *Tegenaria agrestis* show helix (pink color), beta strands (yellow) turns (blue) and white coil regions.

conservation and high sequence similarity. Homology modeling is only a viable technique because it produces models that can be used for further research. Homology modeling helps in predicting the 3-D structure of a macromolecule with unknown structure (target) by comparing it with a known template from another, structurally highly similar, macromolecule. The structure of the target protein is structurally similar with the template if both the target and template sequences are similar. In general, 30% sequence homology is required for generating useful models. Here, the sequence alignment score was 44 as calculated by ClustalW (http://www.ebi.ac.uk/cgi-bin/clustalw/).

In our study, based on the results obtained from mGenTHREADER program, the X-ray structure of the FERREDOXIN (1FCA) of Clostridium acidurici were selected as templates. MODELLER was used for building the model and global energy minimization. The sequence was obtained from sequence database and was submitted to blastp search. After the BLAST analysis, PROCHECK was used to validate the model. The total energy values of the predicted 3-D model were calculated as 98.0% of Ramachandran plot (Figure 1A) value in 30 and 40 steepest descents and conjugate gradient, respectively. Based on analysis on 118 residues of resolution of at least 20 Å and R factor no greater than 30%, a good quality model would be expected to have over 90% in the most favoured regions.

The refined model was analyzed by different protein analysis programs including PROCHECK for the evaluation of the Ramachandran plot quality, and WHATIF for the calculation of packing quality. This structure was found to be satisfactory based on the above results. The predicted 3-D model of the Paralytic insecticidal toxin (ITX1) of *Tegenaria agrestis* will be very useful in wet laboratory while studying the real structure of the protein.

Conclusions

The structure of Paralytic insecticidal toxin (ITX1) of *Tegenaria agrestis* is important for establishing its molecular function. However, a three dimensional structure is not available as yet at PDB. We developed a homology model for Paralytic insecticidal toxin (ITX1) *Tegenaria agrestis* using MODELLER. The model was further analyzed for residue solvent accessibility in establishing its molecular function. Solvent accessible surface area (ASA) analysis of the Paralytic insecticidal toxin (ITX1) model showed that known key residues playing important role in active site for ligand binding and metal ion binding are buried and not accessible to solvent. The analysis highlights the importance of solvent exposed catalytic residues in molecular function.

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