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COMPUATATIONAL ANALYSIS AND STRUCTURAL CHARECTERIZATION OF ODORANT-BINDING PROTEIN, *BOMBYX MORI*

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ABSTRACT

The period of Computational biology play a broad position in the characterization of proteins, molecular modeling and drug studies. In this study, proteomic tools and computational methods are used to characterize the 7-odorant binding proteins of *Bombyx mori*, which is retrieved from the Swiss-prot Database. Odorant binding proteins are considered to be responsible for the transport of pheromones and also cell signaling. It is present in the olfactory receptors of *B. mori*. Through sequence analysis, we found the similar sets of Protein sequences (hits) and through structural analysis, we could characterize the protein by primary as well as the secondary structure analysis. Subcellular location prediction helped to find location assignment of 7 odorant binding proteins. Finally through homology modeling method we modeled 7 odorant binding protein for further future Perspectives. Modeled quality has checked using Ramachandran plot analysis.

KEYWORD: 7-odorant binding proteins and *Bombyx mori*.

1. INTRODUCTION

The silkworm *Bombyx mori* is the most studied organism in communication of insects and also has important economic value for silk production.[1,2] It has been used as a model insect in genetics and physiology based studies, it also helps in producing massive silk protein during last stage of larval development[3, 4, and 5] Recent studies and success in whole-genome sequencing of *B.mori* characterize significant change in *B. mori* genetics.[6] A number of

silkworms' databases provide genome sequences, expressed sequence tags and cDNAs thus; silkworms are helpful representation for comparing sequences within the same type of genus.[7] Also for the in vivo evaluation of human protein-protein interactions, an established transgenic silkworm strain has been developed.[8]

Odorant-binding proteins (OBPs) play major roles in the transport of pheromones in the silkworm *B. mori* similar to other insects.[9] It is small polypeptides seen in the olfactory neuronal perireceptor space.[10-13] The OBPs are subdivided into three subfamilies in silkworm *B. mori*; general odorant-binding proteins (GOBPs), pheromone-binding proteins (PBPs), and antennal-binding proteins (ABPs).[14] There is no homologous similarity between OBPs of vertebrate and insects sequence and its structure.[15] In previous years, more than 400 odorant binding proteins have been reported from 40 insects; which belongs to 10 different classifications. Among them 150 OBPs are from 34 different species of Lepidopteron families.[16,17] Their significance in mediating pheromone detection has been confirmed with a number of researches.[18]

Hence understanding of odorant-binding protein is important for survival of *B. mori*, so we presented wide range analysis of sequence, structure and homology based modeling. The sequence analysis helps to provide general information such as features, structure, function and evolution by comparing other biological databases. The protein characterization through primary and secondary structure analysis gives information about number of amino acid, molecular weight, frequency of amino acids and also details about alpha helix, beta turn and coils. The previous study implies that three dimensional structural knowledge of odorant binding protein will give a clue on important potential residues and it mechanism. So we modeled 7 odorant binding proteins using homology modeling.

2. MATERIALS AND METHODS

Seven odorant-binding proteins sequences of *B.mori* were taken from the Swiss-Port/UniProtKB database[19] for predicting the sequence, structural, functional and homology series.

2.1 Sequence Analysis

Sequence analysis of 7 odorant binding proteins was carried out using CLC Sequence Viewer.[20] CLC Sequence Viewer includes database searches, Graphical Views, Restriction Sites and can import wide formats as well.

2.2 Structure analysis

Primary structure analysis was carried out using ProtParam.[21] This is a tool that allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence. The secondary structure analysis was done using SOPMA (Self-Optimized Prediction method With Alignment tool).[22]

2.3 Protein subcellullar prediction

Predicting sub cellular localization from FASTA sequence of proteins will be helpful for figuring out protein functions. Here protein subcellullar prediction have done using Euk-mPLoc 2.0[23] and TargetP.[24] Euk-mPLoc2.0 can be used to predict the sub cellular localization of proteins from their 22 different location sites. TargetP gives the information about secretory pathway, mitochondria and chloroplasts and other sequence properties.

2.4 Homology modeling

Three-dimensional (3D) structures can give deep understanding in the molecular level of protein function and it allows design of site-directed mutagenesis, mutations related studies or the structure based inhibitor design. Here for modelling three dimensional structures, we have used homology modeling and it was carried out using SWISS-MODEL.[25] Each model was built on the basis of specific template structure from PDB database. Modeled structures were refined using ModRefiner.[26] PROCHECK[27] was used for analyzing stereochemistry of proteins.Visualisation of modeled structures was done using Chimera.[28]

3. RESULTS AND DISCUSSIONS

3.1 Sequence analysis

For doing sequence analysis we have downloaded 7 odorant binding protein such as B8ZWK1, B8ZWK2, B8ZWK3, B8ZWK4, B8ZWK5, B8ZWK6, B8ZWK7. All the consent sequences were aligned to study the residues which were highly conserved among all the proteins. Conserved residues are shown with their corresponding symbols while the highly variable amino acids are denoted by “x” symbol. Sequences belonging to the all seven proteins were constructed using the UPGMA method in CLC work bench software as shown in figure 1.

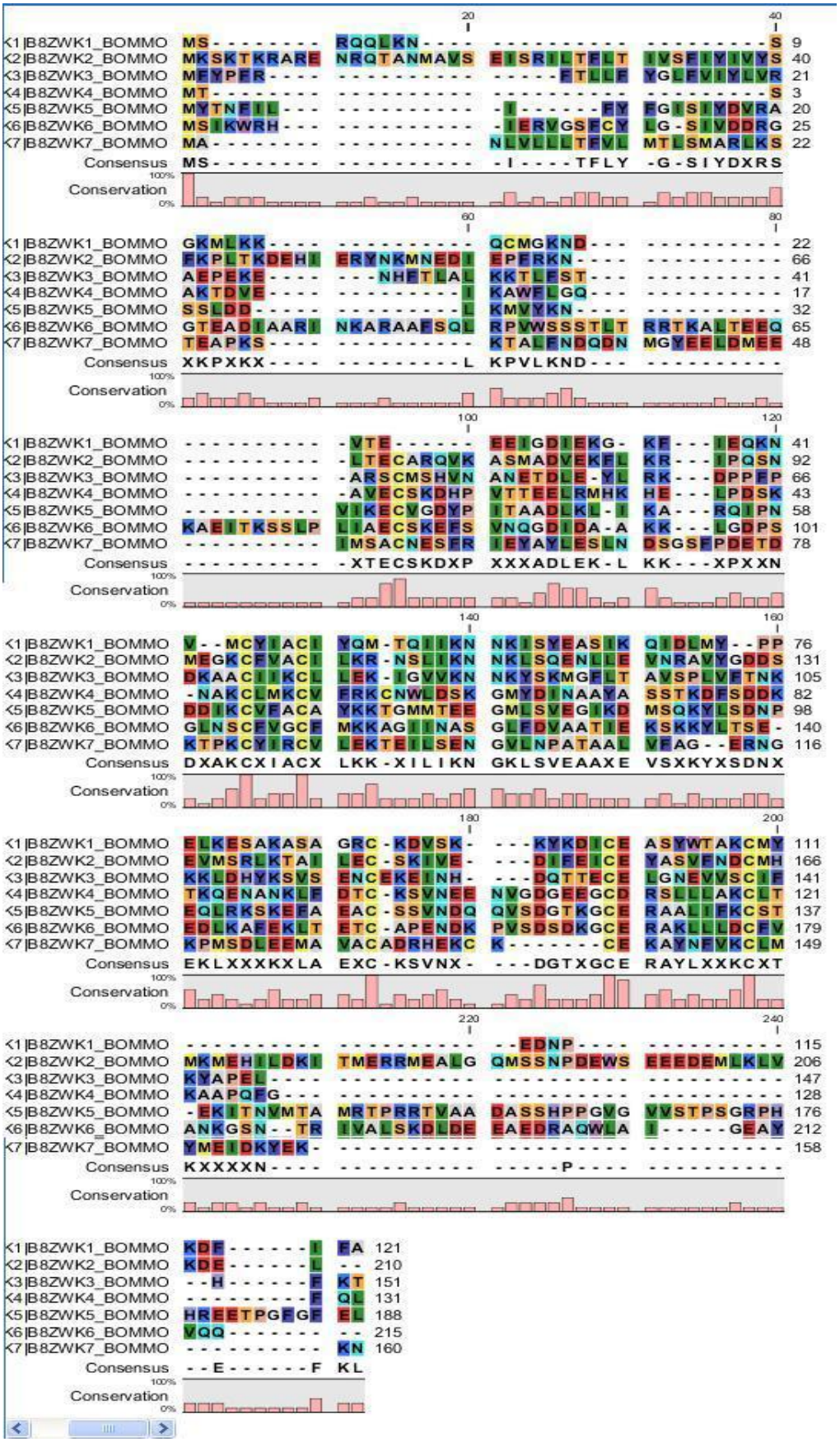

Fig 1: Multiple Sequence Alignment Analysis of 7 odorant-binding proteins using multiple sequence alignment using CLC Workbench.

3.2 Structural characterization

3.2.1 Primary Structure Analysis-ProtParam Tool

Here primary analysis of odorant protein was done using ProtParam Tool. We found number of amino acid, molecular wt, Theoretical PI, Extension Coefficient, instability index and Gravy, and also we could see amino acid composition, atomic composition and also the total number of atoms as shown in Table 1.

Table1: Primary Analysis of 7 odorant-binding proteins using ProtParam Tool.

Uniprot Id	No of A. acid	Mol.wt	Theo. PI	Ext.Coeff	Instability Index	Aliphatic index	GRAVY
B8ZWK1	121	14004.30	8.66	15930	51.07	65.37	-0.665
B8ZWK2	210	24612.50	5.80	12950	65.17	83.10	-0.474
B8ZWK3	151	17502.3	8.47	10805	53.03	81.32	-0.232
B8ZWK4	131	14719.69	5.92	13980	52.06	60.38	-0.643
B8ZWK5	188	20852.92	6.82	10430	33.07	73.14	-0.295
B8ZWK6	215	23550.69	5.67	20970	43.78	80.88	-0.369
B8ZWK7	160	18176.89	4.74	10430	48.29	75.06	-0.341

3.2.2 Secondary structure analysis

Secondary structure analysis was done using SOPMA using parameters number of conformational states as 4, Similarity threshold as 8 and Window width as 7. The result given detailed idea about alpha helix, beta turn and random coil. Here we analyzed 7 odorant binding protein and the results are given Table 2.

Table 2: Secondary analysis result of 7 odorant binding protein.

Uniprot ID	Alpha helix	Beta turn	Random coil
B8ZWK1	63.64%	9.09%	18.18%
B8ZWK2	75.71%	1.90%	14.29%
B8ZWK3	49.67%	8.61%	25.83%
B8ZWK4	48.09%	4.58%	44.27%
B8ZWK5	41.49%	10.11%	32.45%
B8ZWK6	52.09%	13.02%	22.33%
B8ZWK7	56.25%	8.12%	29.38%

3.2.3 Protein subcellullar prediction

Here from the analysis we could analyze that all seven proteins are extracellular proteins. B8ZWK2 are present in both nucleus and extracell. B8ZWK6 present in cytoplasm as well as extracell. Here mTP represents mitochondrial targeting peptide, SP, a signal peptide, S represents secretory pathway. The results are shown in table 3.

Table 3: Subcellular prediction result of 7 odorant binding protein.

Uniprot ID	Subcellular location	mTP	SP	other	Loc	RC
B8ZWK1	Extracell	0.139	0.048	0.894	_	2
B8ZWK2	Extracell. Nucleus	0.603	0.2	0.195	M	3
B8ZWK3	Extracell	0.049	0.875	0.037	S	1
B8ZWK4	Extracell.	0.039	0.207	0.83	_	2
B8ZWK5	Extracell.	0.071	0.729	0.299	S	3
B8ZWK6	Cytoplasm. Extracell.	0.063	0.216	0.809	_	3
B8ZWK7	Extracell	0.081	0.93	0.029	S	1

3.3. Homology modeling, Refining and Ramachandran plot analysis

Homology modeling of 7 odorant binding protein such as B8ZWK1, B8ZWK2, B8ZWK3, B8ZWK4, B8ZWK5, B8ZWK6, and B8ZWK7 was carried out using SWISS-MODEL. SWISS-MODEL is a fully automated protein structure homology-modeling server hence the server selected templates automatically. We refined the 7 proteins using ModRefiner. After getting refined model we checked the quality through Ramachandran plot analysis using procheck. As the procheck results were excellent we could say that the model quality was good. The modeled structures and procheck analysis results are given in Fig 2 and Table 4 respectively.

Table 4. Procheck result of proteins 7 odorant binding.

Uniprot ID	Most favored regions	G-factors
B8ZWK1	91.7%	-0.41
B8ZWK2	92.5%	-0.62
B8ZWK3	94.4%	-0.64
B8ZWK4	99.1%	-0.52
B8ZWK5	95.5%	-1.75
B8ZWK6	93.7%	-0.68
B8ZWK7	93.9%	-0.75

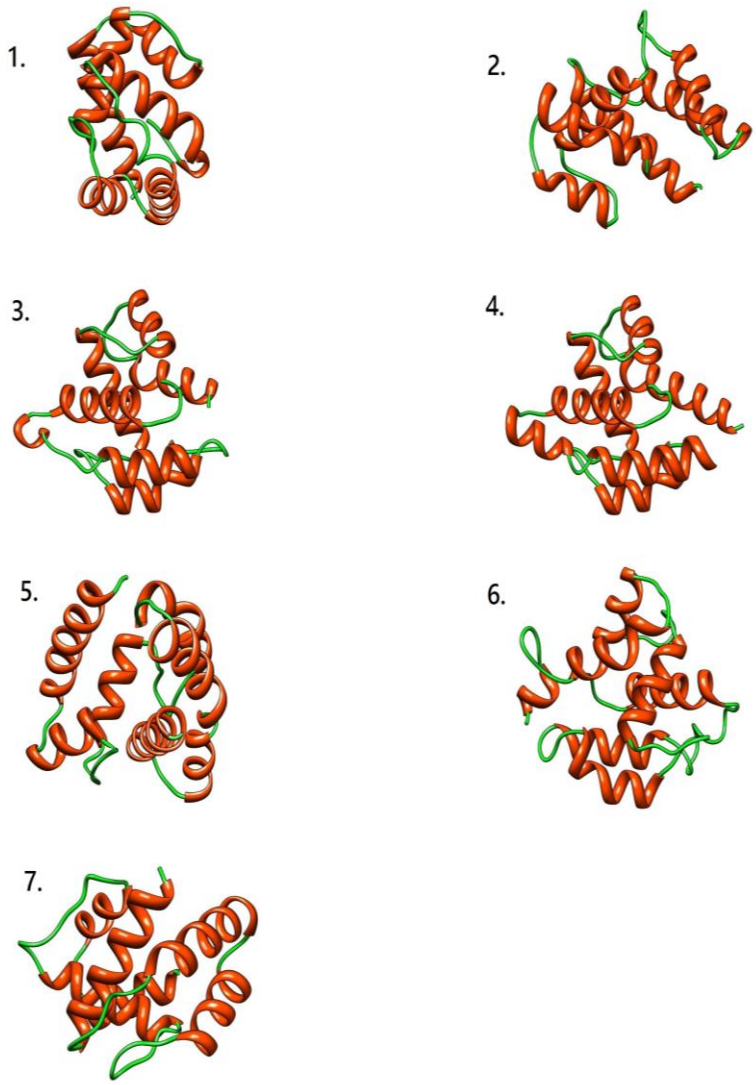

Fig 2: **Modeled image of 7 odorant binding protein and orange color represents helix and green coils.**

5. CONCLUSION

The present research work has provided a good basis for two kinds of expression analysis of odorant protein through sequence analysis. The result visualizes the type of protein, its structure and function analysis. Further studies help to identify the active binding site of odorant binding proteins. Understanding the structural characters can pave way for design of novel inhibitors, lead modification and structure based drug discovery. Keeping this in mind, the present work we analyzed 8-odorant proteins, which has a key role in cell signaling. In this study, we construed 3D models of *Bombyx Mori* odorant binding proteins on the basis of sequence and structural similarity.The quality of the structure predicted using Ramachandran plot analysis.

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