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Research Article

COMPARATIVE MODELLING OF TERTIARY STRUCTURES OF TWO VIRAL PROTEINS FROM *BOMBYX MORI*

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ABSTRACT

The non-diapausing silkworm *Bombyx mori* is exploited both as a powerful biological model system and also as a tool to convert leaf protein into silk. Silkworm often suffers from viral infections causing heavy losses to the economy of the silk industry. One of the main virus pathogens is a nucleopolyhedrovirus, *B. mori* NPV (BmNPV). The present study is extended to Homology modelling of two important proteins- DNA-binding protein (DBP) and Late expression factor II (LEF-II) extracted from the UNIPROKB/SWISSPROT database. Further, the verified 3D structures were deposited to PMDB (PMDB Ids: PM0074795 and PM0074790) and concerned for characterization using structure and sequence based function prediction tools. The investigation will be helpful in understanding the molecular aspects of antiviral immunity in silkworm.

Keywords: Antiviral activity, *Baculoviridae*, BmNPV, Homology modeling.

INTRODUCTION

The silkworm *Bombyx mori* has been exploited as a silk producer in the silk industry for thousands of years. Recent success of transgenesis of the silkworm has opened new prospects for this insect species (Tamura *et al*., 2000). The *B. mori* nucleopolyhedrovirus (BmNPV) is one of the most harmful viruses in the sericulture industry, often causing severe economic losses (Ponnuvel *et al*., 2003). It is well known that insects are targets of viruses and it is true especially in the case of lepidopteran insects, where the number of species found to be infected by viruses is twice that compared to other holometabolic orders (Martignoni and Iwai, 1986). The mechanism by which the insect resists viral infections, recognizes infected cells and recruits immune cells to the infective foci or clears infected cells is poorly understood (Popham *et al*., 2004). Insects seemingly lack any adaptive immune responses that operate analogously to the well-documented antibody or histocompatibility adaptive immune responses as in vertebrates (Hoffmann, 2003).

Nucleopolyhedroviruses (NPVs), members of a genus of the family *Baculoviridae*, have large, circular, double-stranded DNA genomes and are pathogenic for invertebrates, particularly insects of the order Lepidoptera. One of the insects most frequently used for studies of baculovirus infection is the silkworm, *B. mori*. Historical records indicate that *B. mori* was domesticated from the wild silkmoth, *B. mandarina*, several thousand years ago (Yoshitake, 1988). Subsequently, *B. mori* was propagated on a large scale and utilized for silk production in China, Japan and Europe. A recurrent threat to this industry was the infection of colonies by viral diseases. The genome of the nucleopolyhedrovirus (NPV) pathogenic for *B. mori* (Bm) was sequenced which is 128413 nucleotides long with a GC content of 40% and contained 136 open reading frames (ORFs) encoding predicted proteins of over 60 amino acids (*Sumiko et al.,* 1999).

Although, there is an availability of sequence information for a few proteins of Nucleopolyhedroviruses (NPVs) from *B. mori* the biochemistry and molecular mechanism of their functions are still not very well understood due to lack of their species specific structural information. Therefore, this study was extended to sequence analyses and 3D structure prediction (Zemla *et al.,* 1999) of two impotent viral proteins of *B. mori*,

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namely, DNA-binding protein (DBP) and LEF-II (LEF-II) from *B. mori* nucleopolyhedrovirus (BmNPV).

MATERIALS AND METHODS

Sequence analyses

The study was extended to data mining and sequence analysis of DBP and LEF-II from *Bombyx mori* nucleopolyhedrovirus (BmNPV) (Maeda *et al.,*1991; Gomi *et al.,* 1999). The amino acid sequence for each protein was extracted from EBI-SWISSPROT protein knowledge base (Boeckmann *et al.,* 2003; Apweiler *et al.,* 2004). The statistical analyses were performed using CLC Protein Workbench (http://www.clcbio.com), PrptParam (Gasteiger *et al.,* 2005) and Protein Calculator v3.3 (http://www.scripps.edu/~cdputnam/protcalc.html). The important calculations for the amino acid composition, atomic composition, theoretical pI, molecular weight, formula, extinction coefficients, half-life, instability index, aliphatic index, hydrophobicity, charge vs pH, etc. were carried out under sequence analysis.

Comparative Modeling

WU BLAST (Altschul *et al.,* 1997), NCBI BLASTp (Altschul, 1997) and FASTA (Pearson and Lipman, 1988; Pearson, 1990 &1991) searches were performed independently with PDB (Kouranov *et al.,* 2006; Berman *et al*., 2007) for selection of suitable template .The significance of the BLAST results was assessed by expect values (e-value) generated by BLAST family of search algorithm (Altschul, 1991). The target-template alignment (Lassmann and Sonnhammer, 2005) were carried out using clustalW (Higgins *et al*., 1994), 3DCoffee (O'Sullivan *et al.,* 2004) T Coffee (Notredame *et al.,* 2000) and Modeller (Fiser *et al*., 2000) programme. Comparative (Homology) modeling was conducted by using Modeller program (Marti-Renom *et al.,* 2000). The loop regions were modeled using MODLOOP server (Fiser and Sali, 2003), which uses Modeller9v1 package (Marti-Renom *et al.,* 2002). The disordered regions were predicted steriochemically using Modeller .The final 3D structures with all the coordinates for both the proteins were obtained by optimization of a molecular probability density function (pdf) of MODELLER (Fiser and Sali, 2003). The molecular pdf for homology modeling was optimized with the variable target function procedure in Cartesian space that employed the method of conjugate gradients and molecular dynamics with simulated annealing (Sali and Blundell, 1993).

Model verification and deposition

The final 3D structure for each viral protein was evaluated (Giorgetti *et al*., 2005) by ERRAT (Colovos and Yeates, 1993), ProCheck (Laskowski *et al.,* 2003), WHAT_CHECK (Hooft *et al*, 1996), PROVE (Pontius *et al.,* 1996) and Verify 3D (Luthy *et al.,* 1992; Eisenberg *et al.,* 1997) packages. After fruitful verification the coordinate files were successfully deposited to PMDB, University of Rome (Tiziana *et al*., 2006). All the graphic presentations of the 3D structures were prepared using Chimera (Pettersen *et al.,* 2004) and Rasmol (Sayle *et al.,* 1995).A preliminary investigation of function for both the proteins were performed from their respective 3D structures (Laskowski *et al.,* 2005a) using ProFunc (Laskowski *et al*., 2005b). A number of databases like PFam, PROSITE, PRINTS, ProDom, InterProScan (Zdobnov and Apweiler, 2001) also used for functional characterization.

RESULTS AND DISCUSSION

Sequence analyses

Based on WU BLAST and EBI's FASTA results, PDB IDs 2AWR (Chain B, Protamine-DNA complex 1, Identities 37.26%) and 2JBS (Chain A, Structure of the monooxygenase component of p- hydroxyphenylacetate hydroxylase from *Acinetobacter baumannii*, Identities 45.16 %) respectively are considered to be the best templates for modeling of DBP and LEF-II from BmNPV. The DBP does not contain any Trp residues.The extinction coefficient for DBP is 10430 (Abs 0.1% (=1 g/l), 1.291, assuming ALL Cys residues appear as half cystines) which is in units of M^{-1} cm^{-1}, at 280 nm measured in water. The instability index (II) for DBP is computed to be 185.72.This classifies the protein as unstable. The Extinction coefficients for LEF-II are 11835 (Abs 0.1% (=1 g/l) 0.902, assuming ALL Cys residues appear as half cystines) and 11460 (Abs 0.1% (=1 g/l) 0.874, assuming NO Cys residues appear as half cystines). The instability index (II) for LEF-II is computed to be 33.83.This classifies the protein as stable. The important statistical parameters are as listed in Table 1.

The theoretical Structures

The resultant 3D structures of DBP and LEF-II from BmNPV were based on the coordinates from PDB Ids 2AWR and 2JBS respectively, obtained through WU BLAST and EBI's FASTA and target-template alignment results. The 3D structures of DBP and LEF-II from BmNPV have been assigned PMDB Ids PM0074795 and PM0074790 respectively (Figure 1) for the coordinate entry. The predicted 3D structures were found to be statistically significant by the structure verification programs. The ProMotif (Hutchinson *et al.,* 1996) searches reveled that the DBP has 1 sheet, 1 beta hairpin, 1 beta bulge, 2 strands, 4 helices, 6 beta turns and 1 gamma turn. The LEF-II has 1 sheet, 1 beta hairpin, 2 strands, 10 helices, 8 helix-helix interacs, 3 beta turns, 1 gamma turns (Figure 2).

Table 1. Protein statistics.

Protein statistics	DNA-binding protein	Late expression factor II
Sequence type	Protein	Protein
Number of amino acids	64	112
Organism	BmNPV	BmNPV
Gene name	None	LEF- *II*
SWISS PROT AC number	P24649	O92404
Sequence Modification date	October 31, 2006	October 31, 2006
Molecular weight	7946.9 Da	13115.3 Da
Theoretical pI	12.35	9.56
Total number of negatively charged residues (Asp + Glu)	0	10
Total number of positively charged residues (Arg + Lys)	26	20
Formula	$C_{329}H_{562}N_{142}O_{90}$	$C_{577}H_{936}N_{172}O_{159}S_9$
Total number of atoms	1123	1853
Ext. coefficient	10430	11835
Instability index	188.47	33.83
Aliphatic index	25.94	85.27
Grand average of hydropathicity (GRAVY)	-1.955	-0.466
Total number of Hydrophobic residues (A,F,G,I,L,M,P,V,W)	13	43
Total number of Hydrophilic residues (C,N,Q,S,T,Y)	26	32

Table 2. Summery of predicted function by ProFunc.

Summary of predicted function		DNA-binding protein	Late expression factor II
Protein name terms		50s (6.05) ribosomal (4.35) 50s ribosomal (3.64) bound (3.25) a-binding (0.70) protamine-dna (0.70)	Late expression (12.18) factor (11.64); late Expression factor (10.98) lef-11 (4.90);
Gene Ontology (GO) terms	Cellular component	-	Mitochondrion (0.80) cell (0.80) cell part (0.80) intracellular (0.80
	Biological process	-	Viral infectious cycle (19.07); viral life cycle (19.07); cellular physiological process (19.07)
	Biochemical function	-	RNA binding (0.80) RNA-directed RNA polymerase activity (0.80); binding (0.80) nucleic acid binding (0.80

Procheck verification proved that the model is of good quality (judged by Ramachandran Plot) (Figure 3) (Ramachandran and Sasisekharan, 1968). ERRAT verification reveled that the overall quality factors for DBP and LEF-II are 97.778 and 96.939 respectively (Figure 4). The possible ProFunc program and InterProScan function prediction results are as listed in the Table 2. Matching folds for LEF-II detected by SSM and Dali and 3D functional template searches showed significant structural matches with the template 1lr0 (RMSD 1.26Å, Z-score 4.4. SSE 3. Matches to the Superfamily HMM library reveled that the DBP of BmNPV is a Arginine-rich, Basic viral nucleocapsid protein, responsible for DNA condensation during packaging of the nucleocapsids, phosphorylated in infected cells. DBP binds preferentially to ssDNA and is capable of unwinding duplex DNA. The BmNPV open reading frame (ORF) encoding DBP (*dbp* gene) is a homolog of A*cMNPV* ORF25, whose product has not been identified so far. LEF-II belongs to the baculoviruses LEF-11 family, Involved in late/very late gene activation.

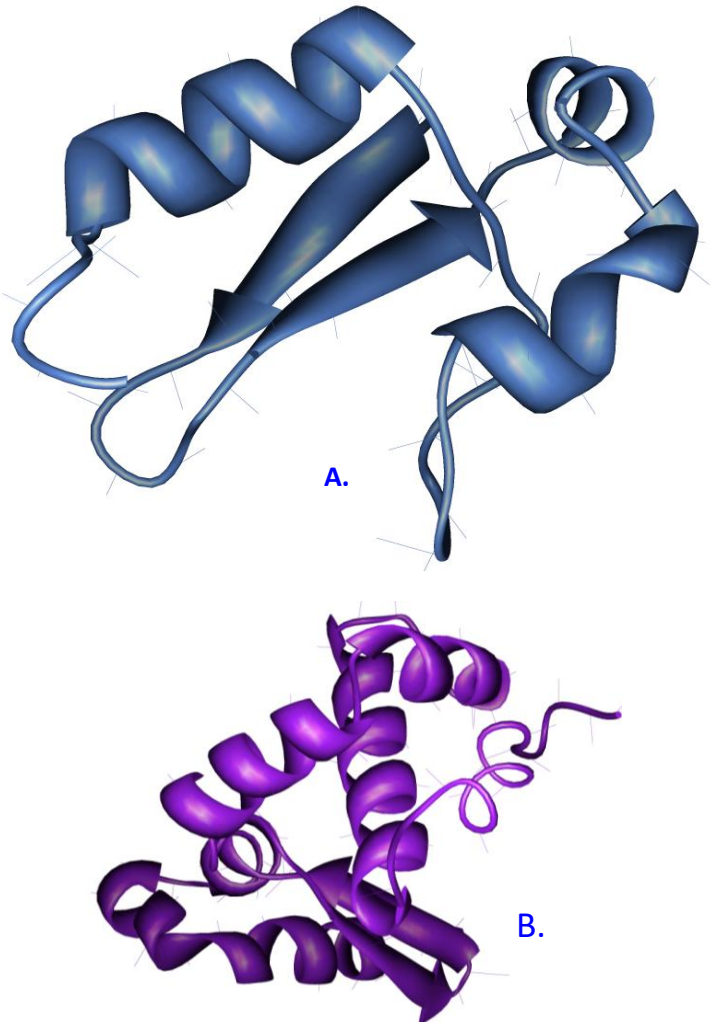


Figure 1. The predicted 3D structure of A. DNA-binding protein (PMDB ID: PM0074795), B. LEF-II (PMDB ID: PM0074790) from BmNPV displayed by UCSF Chimera.

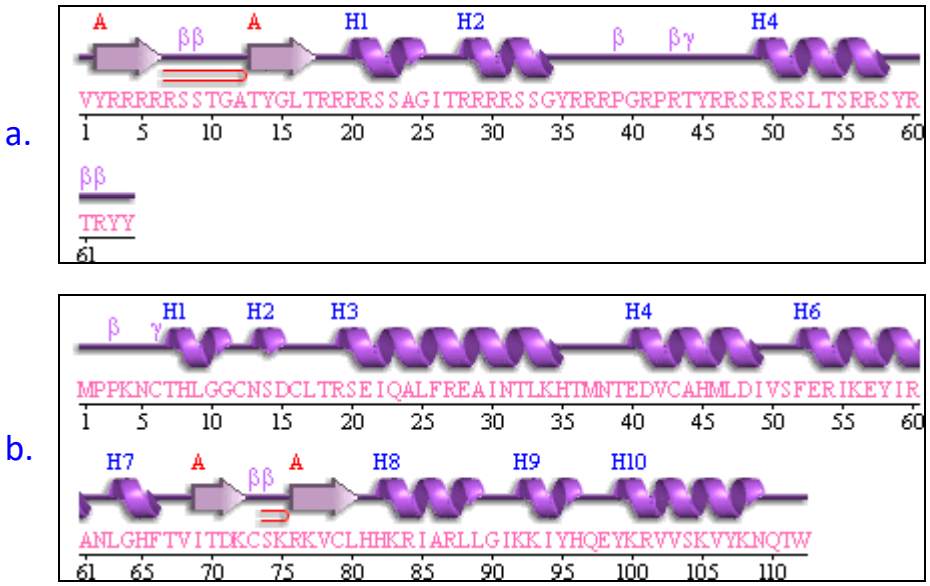
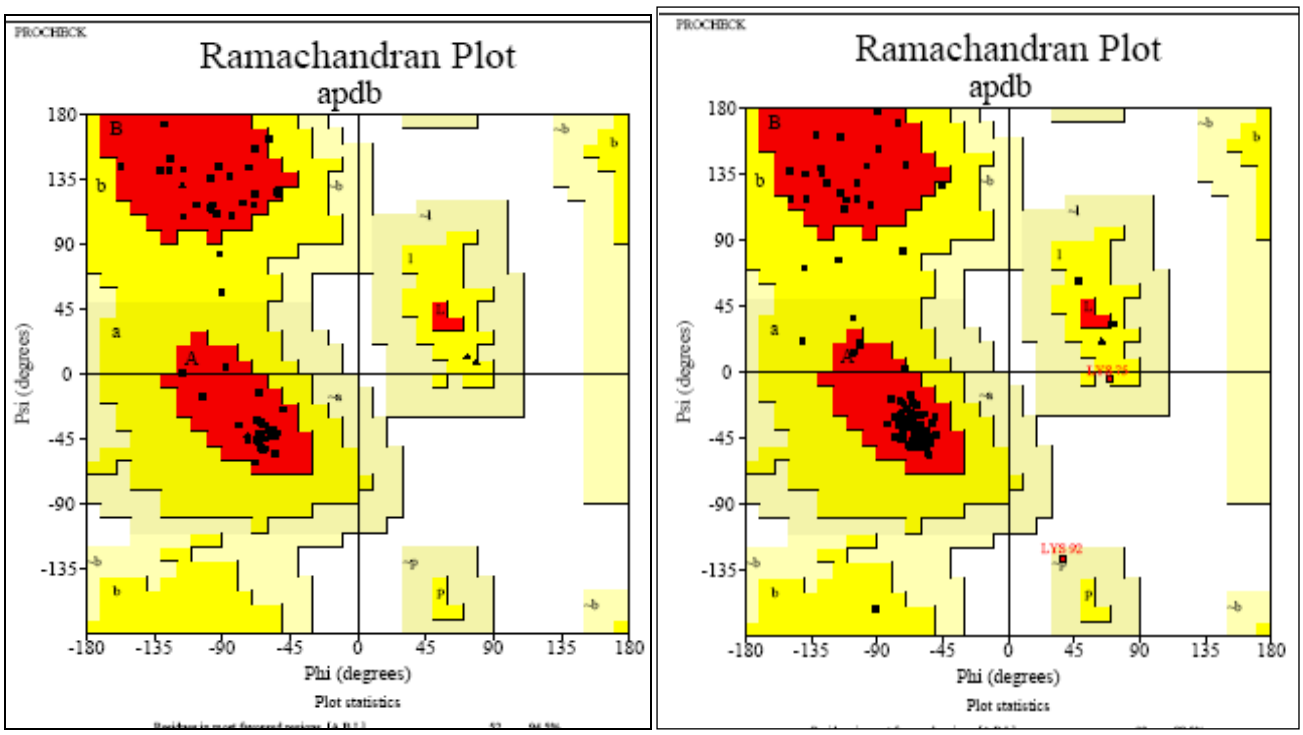
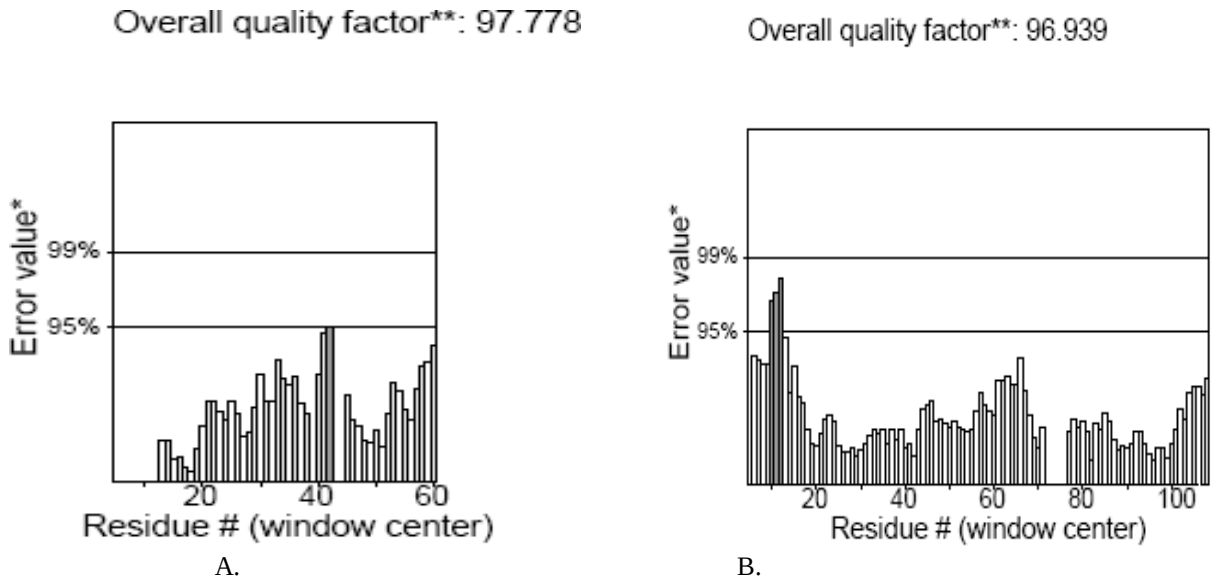


Figure 2: Secondary structure assignment of the predicted three-dimensional models of a. DNA-binding protein, b. LEF-II.



a. b.

Figure 3. Ramachandran analysis of the backbone dihedral angles PSI(Ψ) and PHI(Φ) for the final structure of a. DNA-binding protein, b. LEF-II calculated with ProCheck(Laskowski *et al.*,2003). Red region represents the most favored region, yellow = allowed region, light yellow = generously allowed region, white = disallowed region.



A. B.

Figure 4: Structure validation results showing Overall qualiy for 3D Models of A. DNA-binding protein, B. LEF-II (ERRAT2 Verification). **Abbreviations:** 3D –Three Dimensional; BmNPV– *Bombyx mori* nuclear polyhedrosis virus; DBP–DNA-binding protein; EBI–European Bioinformatics Institute; FASTA–Fast Analysis; LEF-II–Late expression factor II; NCBI–National Center for Biotechnology Information; PDB–Protein Data Bank; PROVE– Protein Volume Evaluator at UCMB; PMDB–Protein Model Data Base; RMSD-Root Mean Square Deviation; WU-Blast2–Washington University Basic Local Alignment Search Tool.

The modelling of DBP and LEF-II from BmNPV gains importance for the structural biologist and even to the Seribiotechnological researchers. Comparative (Homology) modelling is the most successful structure prediction method (Moult *et al*., 2003) that focuses on the use of structural templates derived from known structures to build an all-atom model of a target. Comparative modelling has better prediction accuracy with RMSD 1 to 4 A°. The predicted 3D structures for DBP and LEF-II from BmNPV will definitely help in studying the molecular mechanism of functions and also further wet lab analyses (Peitsch, 2002).

The mulberry silkworm, domesticated and mass reared for several centuries, presumably has weakened immune system which has made the insect highly vulnerable to bacterial and viral infections. Information on the organization and function of the immune system in the silkworm in general and with reference to viral infections in particular is scanty. There are a few recent reports on the presence of antiviral proteins, against some DNA and RNA viruses, which strongly suggests the presence of a functional antiviral immune system in the silkworm. A number of mechanisms operate at different levels, from the site of origin of infection viz midgut through the medium of transport viz hemolymph to the peripheral tissues where virus proliferates, stalling the initiation, propagation and multiplication of the virus. There are a few scanty reports on the host-parasite interaction at each of the three levels but detailed investigations are required before stepping into disease control and also other aspects such as utilization of silkworm as a bioreactor to produce antiviral proteins of interest and having application in human health and welfare.

CONCLUSION

The high economic value of the silk industry further emphasizes the need for determination of disease related protein structure to study the functional specificity and recognition of targets for developing inhibitor molecules against viral pathogens. Therefore, the models presented here can serve as a guide for the allocation of amino acid residues involved in each fold, which is important for further investigations on active sites and molecular mechanism of function. The study was performed for sequence analyses and prediction of 3D structure of DBP and LEF-II from BmNPV using the Comparative (Homology) modelling due to high level sequence identity with the previously solved structures of Protein Data Bank. A series of molecular modelling and computational methods were combined in order to gain insight into the 3D structure.

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