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Cloning, Characterization and Bioinformatic Analysis of the Gene Encoding the Larval Serum Protein 2 in Diapause of the Onion Maggot, *Delia Antiqua*

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ABSTRACT

The full-length cDNA encoding Larval serum protein 2 (LSp-2) in the onion maggot, *Delia antiqua*, was cloned and sequenced by rapid amplification of cDNA ends methods. The result showed that the cDNA was 2203 bp long and the open reading frame (ORF) of 2106 bp encoded 701 amino acid with a calculated molecular weight of 80.5 kDa and an isoelectric point of 5.87. The onion maggot LSp-2 shows highest homology (83%) to that of *Calliphora vicina* at amino acid level. Its signal peptides, domains and structures were predicted and analyzed by using bioinformatic methods. The amino acid sequence of LSP-2 suggests that it would be a typical hexamerin.

Keywords: Delia Antiqua; Diapause; Larval Serum Protein 2; Cloning; Molecular Characterization; Bioinformatic Analysis

1. Introduction

Larval serum protein 2 (LSP-2) belongs to a superfamily of hexameric hemolymph proteins that have been found in all insect species investigated [1]. In holometabolous insects, hexamerins are thought to act mainly as storage proteins that provide energy and amino acids during metamorphosis [2,3]. Expression of *LSP*-2 is restricted to the fat body cells, where it reabsorbs proteins and other macromolecules that have accumulated in the haemolymph during the larval feeding period [5,6]. Roughly half of the cell population survives metamorphosis, indicating a specific degree of differentiation during postembryonic life. Diapause is a developmental strategy widespread among insects and their arthropod relatives. The onion maggot (*Delia antiqua*), a serious pest of onion (*Alliumcepa*), passing over winters and/or summers as diapausing pupae, is an excellent model for diapause research [4]. LSP-2 as a kind of storage proteins is believed to provide with a means for the feeding insect to build reserves of amino acids for use in protein synthesis and energy metabolism during diapause stages.

2. Materials and Methods

2.1. Insects

The colony of *D. antiqua* was reared on an artificial diet at 25˚C with a 16L:8D cycle and relative humidity 50% - 70%. Larvae were maintained at 25˚C with a 16L:8D photocycle to induce SD and 16˚C with 12L:12D to induce WD.

2.2. RNA Extraction and RT-PCR

Total RNAs were extracted with Trizol® reagent (Invitrogen Co., USA) from the pupae according to the manufacturer's instructions. The first -strand cDNA was synthesized from 1 μg of total RNA in 20 μl reaction mixture prepared with RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). One microliter of the reaction mixture was added to 25 μl of PCR reaction system, PCR amplification was performed using one specific primers ku6contig11F (5'-CCACTGGCTTGACTCGCATG-3') and ku399R (5'-GCGGGCTCCAAAGTATTCA-3') (**Figure 1**) based on known EST sequences in SSH cDNA library conservative sequences with the following reaction conditions: 1 min at 94˚C, 30 S at 55˚C, 30 S at 72˚C with 35 cycles, then 10 min at 72˚C.

2.3. RACE Amplification

Specific primers were synthesized for 5' and 3'RACE based on the cDNA sequences obtained from internal amplification. For 5' and 3'RACE, the cDNA was synthesized according to the manufacturer's protocol

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(SMARTer™ RACE cDNA Amplification Kit, Clontech). 5' and 3'-RACE amplification was performed on 2.5 ml of 3'-ready-cDNA with UPM and GSPF. The reaction mixture was subjected to 30 thermal cycles that consisted of 94˚C, 1 min; 65˚C, 1 min; 72˚C, 1 min.

2.4. Cloning and Sequencing

The amplified DNAs were separated on a 1.5% agarose gel and purified using DNA Gel Extraction Kit. DNA fragments were cloned according to the manufacturer's protocol (TOPO TA Cloning Kit for Sequencing). Multiple sequencing reactions were run using both M13F and M13R primers.

2.5. Phylogenetic Analysis

The program MEGA5.0 was used for the phylogenetic tree reconstructions, and Neighbor-joining tree were inferred with the program.

3. Results

3.1. Cloning of Onion Maggot LSP-2 cDNA

The tools provided by the ExPASy Molecular Biology Server of the Swiss Institute of Bioinformatics (http://www.expasy.org) were used for the analyses of DNA and amino acid sequences. Onion maggot *LSP*-2 cDNA contains a 2106 bp open reading frame (ORF) encoding a 701 amino acid precursor protein. The first 21 amino acids are predicted for the sequence of a signal peptide using SignalP Version 3.0 software (http://www.cbs.dtu.dk/services/SignalP). The 5' untranslated region upstream of the transcription start code (ATG) is about 23 nucleotides. The ORF is terminated by a TAA stop code that is followed by a 74bp 3' untranslated region.

3.2. Bioinformatics Analysis

As determined by the use of protparam software (http://web.expasy.org/protparam) the deduced amino acid sequence of 680 residues has a molecular mass of 80.5 kDa and a pI of 5.87. Onion maggot *LSP*-2 was most similar to LSP-2 from *Drosophila melanogaster* and *Calliphora vicina*. Using the SMART software (http://smart.embl-heidelberg.de) found domains (**Figure 1**). Putative glycosylation and phosphorylation sites as well as the conserved motifs ADKDFLXKQK (position 27; Gordadze *et al*., 1999) and TMMRDPMFY (position 480; PROSITE, Bairoch *et al*., 1997), found in several insect hexamerins, were identified in the LSP-2 amino acid sequence [7].

The position of secondary-structure elements was deduced using UniProt (http://www.uniprot.org) (**Figure 2**).

Name	Begin	End	E-value
Pfam: Hemocyanin_N	31	156	6.80E-32
Pfam: Hemocyanin_M	160	421	1.30E-53
Pfam: Hemocyanin_C	427	694	1.30E-89

Figure 1. Predicted domain of LSP-2.

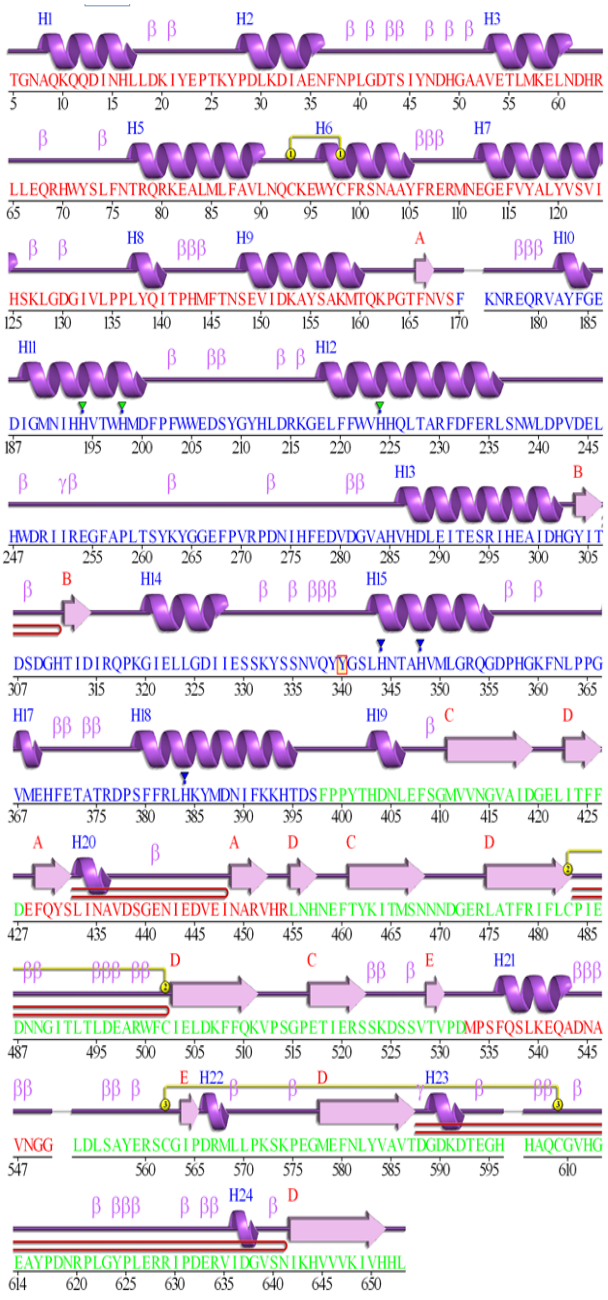

Figure 2. Protein secondary-structure of onion maggot LSP-2.

The position of tertiary-structure elements was deduced using UniProt (http://www.uniprot.org) (**Figure 3**).

3.3. Phylogenetic Analysis

The multiple-sequence alignment of 6 LSP-2s was analyzed by the neighbor-joining method. The tree is rooted by the LSP-2 of *Bomyx mori* (**Figure 4**).

4. Discussion

Here we cloned and characterized the cDNA sequence of the *Lsp*-2 gene and examined the structure of its protein Except the 21 amino acid signal peptide, translation of the LSP-2 ORF yields a polypeptide of 80.5 kDa. During diapause LSP-2 synthesized in the fat body and secreted into the hemolymph is markedly elevated [8-11]. During diapause and metamorphosis LSP-2 may serve as an amino acid store for development of other adult structures. Thus, it is interesting to study the fate of LSP-2 during metamorphosis and post-diapause development respectively in order to fully understand the exact function of this protein during.

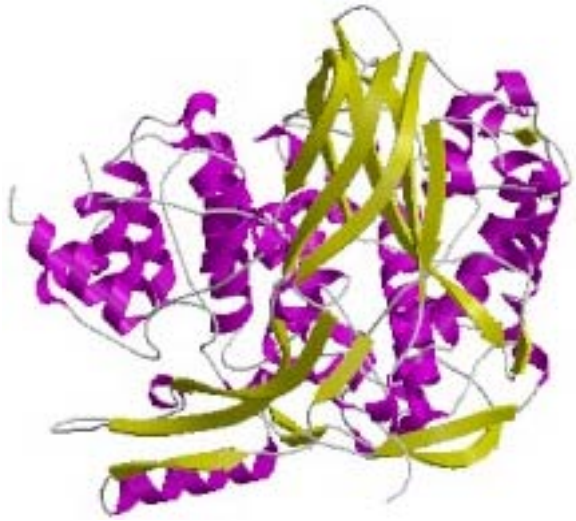

Figure 3. Protein tertiary-structure of the onion maggot LSP-2.

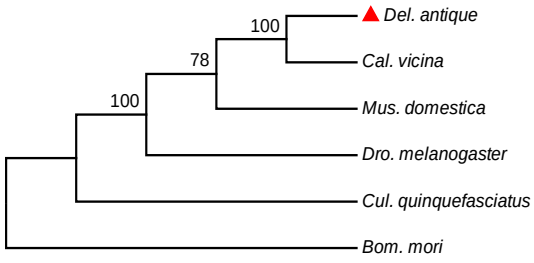

Figure 4. Phylogenetic analysis by NJ trees based on the amino acids sequences of LSP-2s. Branch lengths are proportional to the numbers of amino acid substitutions. GenBank accession number was: *Calliphora vicina* (AAC 24157.1), *Musca domestica* (AAP13346.1), *Drosophila melanogaster* (NP524816.1), *Culex quinquefasciatus* (EDS32906.1), *Bombyx mori* (BAA02093.1).

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