cDNA sequences and mRNA levels of two hexamerin storage proteins PinSP1 and PinSP2 from the Indianmeal moth, *Plodia interpunctella*

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Abstract

In insects, storage proteins or hexamerins accumulate apparently to serve as sources of amino acids during metamorphosis and reproduction. Two storage protein-like cDNAs obtained from a cDNA library prepared from fourth instar larvae of the Indianmeal moth (*Plodia interpunctella*) were cloned and sequenced. The first clone, PinSP1, contained 2431 nucleotides with a 2295 nucleotide open reading frame (ORF) encoding a protein with 765 amino acid residues. The second cDNA, PinSP2, consisted of 2336 nucleotides with a 2250-nucleotide ORF encoding a protein with 750 amino acid residues. PinSP1 and PinSP2 shared 59% nucleotide sequence identity and 44% deduced amino acid sequence identity. A 17-amino acid signal peptide and a molecular mass of 90.4 kDa were predicted for the PinSP1 protein, whereas a 15-amino acid signal peptide and a mass of 88 kDa were predicted for PinSP2. Both proteins contained conserved insect larval storage protein signature sequence patterns and were 60–70% identical to other lepidopteran larval storage proteins. Expression of mRNA for both larval storage proteins was determined using the quantitative reverse transcription polymerase chain reaction method. Only very low levels were present in the second instar, but both mRNAs dramatically increased during the third instar, peaked in the fourth instar, decreased dramatically late in the same instar and pupal stages, and were undetectable during the adult stage. Males and females exhibited similar mRNA expression levels for both storage proteins during the pupal and adult stages. The results support the hypothesis that *P. interpunctella*, a species that does not feed after the larval stage, accumulates these two storage proteins as reserves during larval development for subsequent use in the pupal and adult stages. Published by Elsevier Science Ltd.

Keywords: Indianmeal moth; *Plodia interpunctella*; Insect; Larval storage protein; cDNA; mRNA; Expression; Development; Stored-product pest; Hexamerins

1. Introduction

Insect hexameric larval storage proteins (LSPs) are synthesized mainly during larval development, stored in the hemolymph, and also sequestered in fat body where they serve as sources of nitrogen and amino acids for utilization by pupae and adults during metamorphosis and reproduction (Kanost et al., 1990; Telfer and Kunkel, 1991). These proteins are important for insect development and, therefore, numerous studies concerning their structure, biosynthesis, regulation and evolution have been conducted during recent years (Haunerland, 1996; Burmester 1999, 2001). Phylogenetic analysis showed that they share a common ancestry with arthropod phenoloxidases such as tyrosinases and the hemocyanins of the Crustacea, which are copper-containing oxygen carriers occurring in the hemolymph of many mollusks and arthropods (Burmester et al., 1998; Burmester, 2001). LSPs and arthropod hemocyanins consist of hexameric or multi-hexameric assemblies of subunits of apparent molecular mass of about 75 kDa, which neither contain copper nor bind oxygen. Two classes of LSPs in insects are methionine-rich LSPs and aromatic
2. Insect culture

2.1. Insect culture

Plodia interpunctella, which is a worldwide economically important pest species of stored products (McGaughey and Beeman, 1988).

In the course of studies of the Indianmeal moth designed to identify genes for proteins that are critical for molting during the larval–pupal and pupal–adult transformations, we isolated two cDNA clones from a cDNA library prepared using mRNA from fourth instar larvae. These clones were sequenced to determine their relationship to genes for other proteins previously described in insects. The predicted amino acid sequences of their protein products are related to storage proteins from several species of insects, including methionine-rich, diapause-associated, and juvenile hormone-suppressible storage proteins (Burmester, 1999). As is typical of many LSPs, transcripts for both P. interpunctella proteins increased gradually during larval growth but decreased during the larval–pupal and pupal–adult transformations.

2. Materials and methods

2.2. mRNA purification and cDNA library construction

Approximately 2 g of fourth instar larvae were homogenized in liquid nitrogen. Total RNA was extracted with a denaturing solution containing guanidine isothiocyanate and precipitated with isopropanol (Titus, 1991). The poly(A)–RNA was isolated from total RNA by chromatography on an oligo(dT)–cellulose column (Gibco BRL Life-Technologies, Gaithersburg, MD). Double stranded cDNA was synthesized using 5 µg of poly(A)–RNA as a template, directionally cloned into a UniZAP XR vector phase (ZAP–cDNA synthesis kit, Stratagene, La Jolla, CA), and packaged using the ZAP–cDNA Gigapack II Gold cloning system (Stratagene). Approximately 2 million recombinants were represented in the cDNA library.

2.3. Development of PCR probes

Polymerase chain reaction (PCR) was conducted to develop a probe for use in the cDNA library screen. Lambda DNA of an amplified cDNA library was prepared using phage precipitation and phenol/chloroform extraction procedures after RNase A and DNase I digestions (Titus, 1991). This DNA was used as a template in subsequent PCR amplification. A forward degenerate primer, 5'-TAYTTTYACNGARGAYATHGAYYT-3' (H=A, C or T; Y=C or T; R=A or G; N=A, T, G or C), was designed based on a highly conserved amino acid sequence YFTEDIDL found in many insect LSPs. By using this forward primer and a T7 primer as the reverse primer, a 1700 bp cDNA fragment was amplified from the cDNA library. Fifty µl of the PCR reaction contained 10 mM Tris–HCl pH 9, 50 mM KCl, 1.5 mM MgCl₂, 0.1 mM of each of dNTP, 0.5 µM of each primer, 100 ng of DNA template, 2.5 units of Taq DNA polymerase (Promega, Madison, WI), and an appropriate volume of dd–H₂O. The DNA template was first denatured for 3 min at 94°C and then amplified using 40 cycles of PCR (30 s at 94°C for denaturing, 30 s at 56°C for annealing, and 1 min at 72°C for extension) with final 10-min extension at 72°C.

PCR products were separated by electrophoresis on a 1% low melting point agarose gel, and the DNA fragments of interest were purified using a Wizard PCR DNA PREP kit (Promega, Madison, WI). Purified DNA fragments were ligated with a pGEM–T vector DNA (Promega, Madison, WI) overnight at 4°C. Escherichia coli JM109 competent cells (Promega) were transformed with this ligation mixture and plated on LB/ampicillin/IPTG/X–Gal medium. White colonies were subjected to PCR amplification to confirm the presence of inserts and the expected sizes of fragments. Plasmid DNA was extracted using a Qiagen Plasmid Mini kit (Qiagen, Santa Clara, CA). Sequences of these clones were determined by using an automated DNA sequencer (Applied Biosystem Model 393A). The cDNA sequences of the putative larval storage proteins were confirmed by homology searching of GenBank at the National Center for Biotechnology using the BLASTX protocol (Altschul et al., 1990; Gish and States, 1993).

2.4. cDNA library screen

The cDNA library was plated at a density of ~130 plaque forming units per square centimeter and plates were transferred to nylon membranes (MSI, Westboro, MA). The membranes were hybridized at 65°C (DNA/RNA Hybridization buffer, Sigma, St Louis, MO) with a PCR-amplified larval storage protein cDNA probe labeled with α-32P-dCTP (Amersham, Arlington Heights, IL). Membranes were washed at 55°C for 1 h with three changes of 0.2×SSC (sodium chloride/sodium citrate)/0.1% SDS and then exposed to X-ray film. Positive clones from the first library screen were subjected to PCR amplification and analyzed for the presence of an insert fragment of the predicted size (~1700 bp when amplified with the T7 and the degenerate primers, and
2.5. Cloning and sequencing of the 5'-end of LSP cDNAs

Two different LSP cDNAs, designated as PinSP1 and PinSP2, were obtained from the screening of the cDNA library. PinSP1 clones contained an open reading frame (ORF) coding for a complete larval storage protein-like protein. However, homology search of the GenBank database indicated the ORFs of PinSP2 clones lacked a reasonable methionine start site, and protein sequences encoded by PinSP2 clones were about 5 amino acids shorter at their N-termini than other similar insect LSPs. Repeated cDNA library screenings and PCR amplifications did not generate any longer clones. To obtain the full PinSP2 cDNA sequence, mRNA was purified from third instar P. interpunctella larvae using the PolyATtract mRNA Isolation System (Promega), cDNA ends were synthesized and amplified using the 5'-RACE System (Gibco BRL, Life Technologies) with 3 gene specific primers (Fig. 2) used for reverse transcription and two subsequent semi-nested PCR amplifications. PCR fragments were cloned and sequences were obtained.

2.6. Sequence analysis

The BLASTX program was used to search the sequence database of the National Center for Biotechnology Information Internet server for proteins with amino acid sequence similarity to the PinSPs (Altschul et al., 1990; Gish and States, 1993). The Wisconsin Sequence Analysis Package GCG (Unix version 9.0, Genetics Computer Group, Madison, WI) including Pileup, Gap, Distances and Growtree programs was used to analyze for similarities of insect LSP sequences (gap weight=5, gap length weight=1). Sequence analysis tools of the ExPASy Molecular Biology Server of the Swiss Institute of Bioinformatics were used to process data of deduced protein sequences.

2.7. Quantitative reverse transcription (QRT) PCR analysis of LSP mRNA expression

Procedures for the quantitative determination of the expression levels of two insect LSP mRNAs were modified from those used by Alexandre et al. (1998), Guenther and Hart (1998), Igaz et al. (1998), Freeman et al. (1999) and Zhu et al. (2000). To develop a homogeneous internal standard for QRT-PCR analysis of PinSP1 mRNA expression, the restriction enzyme Bgl II was used to cut out a 248-bp fragment from PinSP1 cDNA (nucleotides 303–550, Fig. 1). The fragments were religated using T4 DNA ligase (Promega) and used to transform E. coli. A plasmid-containing PinSP1 cDNA fragment with a 248-bp deletion was identified by PCR and agarose gel electrophoresis, and subsequently used as the internal standard for QRT-PCR analysis of PinSP1 mRNA expression.

Because of a lack of appropriate restriction enzyme sites, development of the internal standard for QRT-PCR analysis of PinSP2 mRNA levels was completed by inserting a 250-bp P. interpunctella trypsin-like cDNA fragment (Zhu et al., 2000) at an Aat II restriction site (nucleotides 881–886, Fig. 2).

Total RNA was extracted from groups of 20 second-instar larvae, 20 third-instar larvae, 10 early fourth-instar larvae (feeding stage), 10 late fourth-instar larvae (wandering stage), 10 early pupae (green), 10 late pupae (brown–black), and 10 adults by using TRIZol reagent (Gibco BRL Life-Technologies, Gaithersburg, MD), and precipitated with isopropanol. Reverse transcription was conducted with an oligo-dT primer and SuperScript II reverse transcriptase (Gibco BRL Life-Technologies, Gaithersburg, MD). Five µg of total RNA from each sample were used to generate first strand cDNA, which was then diluted with dd-H2O to obtain a final concentration of cDNA equivalent to 0.125 µg of original RNA per microliter.

QRT–PCR was conducted three times with different RNA preparations for each of the stages of P. interpunctella. Specific primers were designed based on unique sequences for each LSP-like cDNA. Primers JHSP13F and JHSP2R3 (sequences are underlined in Fig. 1) were used to amplify a 1048-bp PinSP1 cDNA fragment, and primers JHSP14F1 and JHSP5R3 (sequences are underlined in Fig. 2) were used to amplify a 767 bp PinSP2 cDNA fragment. The concentration range of the internal standard was determined by preliminary experiments for each insect stage. The standard was diluted with distilled water as follows: 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1,000, 5,000, and 10,000 pg/µl. Six concentrations of the internal standard were predetermined to match target mRNA levels for each stage and were used in the QRT–PCR reactions. The PCR reaction mixture contained 1 µl of standard, 1 µl of target cDNA equivalent to 0.125 µg of total RNA, PCR buffer (10 mM Tris-chloride, pH 9.0, 50 mM KCl and 1.5 mM MgCl2), 0.1 mM of each dNTP, 0.25 µl of each primer (0.25 pmole), 1 µl of DMSO, 1.5 unit of Taq DNA polymerase, and H2O in a final volume of 20 µl. The DNA was initially denatured for 3 min at 94°C, and the PCR amplification included 38 cycles of 30 s
Fig. 1. Nucleotide and deduced amino acid sequences of LSP cDNA PinSP1 isolated from *P. interpunctella*. ATG = start codon, TGA = termination codon, AATAAA = polyadenylation signal and ↑ = predicted signal peptide cleavage site. Primer sequences used for cloning and sequencing cDNA are underlined and labeled on the top. Complementary sequences of the reverse primers are indicated by italics. LSP signature sequences are boxed. Two *Bgl* II restriction sites are indicated with bold underlined letters. The sequence has been deposited in GenBank with accession number AF356842.

3. Results

3.1. LSP cDNA sequences

A PCR amplification strategy was employed to develop a probe for LSP cDNA clones using a highly conserved sequence found in several LSPs for designing the forward primer (see Materials and methods section for details). This PCR product was used to screen a λ-ZAP cDNA library from fourth instar larvae of *P. interpunctella*. More than one hundred positive plaques were obtained from a cDNA library screen. PCR verifications using forward degenerate primer from the conserved
Fig. 2. Nucleotide and deduced amino acid sequences of LSP cDNA PinSP2 isolated from *P. interpunctella*. ATG = start codon, TGA = termination codon, ATTAAA = potential polyadenylation signal and ▲ = predicted signal peptide cleavage site. Primer sequences used for cloning and sequencing cDNA are underlined and labeled on the top. Complementary sequences of the reverse primers are indicated in italics. LSP signature sequences are boxed. The *Aat* II restriction site is indicated with bold underlined letters. The sequence has been deposited in GenBank with accession number AF356843.
nucleotides encoding 750 amino acid residues. Pairwise sequence alignment indicated that PinSP1 and PinSP2 shared 59% cDNA sequence identity (1419 nucleotides, gap open=5, gap extension=1).

3.2. Deduced LSP amino acid sequences

The amino acid sequences of the proteins deduced from PinSP1 and PinSP2 cDNAs had 765 and 750 amino acids, respectively. Pairwise sequence alignment of these two amino acid sequences revealed that these proteins shared 44% amino acid sequence identity (330 residues, gap open=5, gap extension=1). Based on homology searching of the GenBank database, both of these sequences were closely related to several LSPs with names such as methionine-rich storage proteins, diapause-associated proteins, or juvenile hormone-suppressible proteins (Table 1 and see below).

The predicted molecular masses for PinSP1 and PinSP2 were 90.4 and 88.0 kDa, respectively. These proteins apparently contained signal peptides of 17 and 15 amino acids, respectively. At physiological pH, PinSP1 would contain 110 positively charged residues (59 arginines and 51 lysines, 14% of the total amino acids) and 102 negatively charged residues (69 aspartic acids and 33 glutamic acids, 13%), which is consistent with a theoretical pI of pH 8.7. PinSP2 would have 108 positively charged residues (52 arginines and 56 lysines, 14%), 102 negatively charged residues (73 aspartic acids and 29 glutamic acids, 14%), and a theoretical pI of pH 8.5. Other major amino acids included 110 leucine residues (14%) and 76 valines (10%) for PinSP1, and 79 leucines (11%) and 65 valines (9%) for PinSP2. Twenty-five methionine residues made up about 4% of the total amino acids in PinSP1 and 46 methionines made up 6% in PinSP2, levels typically found in methionine-rich LSPs. Seventy-nine and 76 aromatic residues (phenylalanine, tryptophan and tyrosine) compose approximately 10% of the total amino acids in both proteins.

ScanProsite analyses (see http://ca.expasy.org/tools/scnpsite.html) revealed that PinSP1 and PinSP2 were arthropod hemocyanin-like proteins, which contained two signature sequence patterns (Linzen, 1989; Willott et al., 1989; Jones et al., 1990; Burmester, 1999). The LSP signature-1 motif is Y[F, Y or W]X, E or D[L, I, V or M]XNXXXXXXHXXXP. The corresponding sequence in PinSP1 was YFTEDIDLNTYYYYFHVDYP at positions 227–246 (Fig. 1) and the one in PinSP2 was YFTEDIDLSTYLMHYMP at amino acids 230–249 (Fig. 2). In PinSP2 a conserved asparagine residue in the insect LSP signature-1 motif was substituted for by a serine residue (underlined and in bold face). This motif also included a conserved histidyl residue that acts as a ligand for a copper ion in the hemocyanins and contains the highly conserved sequence, YFTEDIDL, which was the basis for the design of the forward degenerate primer used in our PCR experiments. The LSP signature-2 motif is TXXRDPX[F or Y][F, Y or W] and the corresponding sequences in PinSP1 and PinSP2 were TALRDPVFY at positions 424–432 (Fig. 1) and TCLRDPVYW at amino acids 422–430, respectively (Fig. 2).

3.3. Comparison of insect LSP amino acid sequences

By using the homology searching software of GenBank, PinSP1 was found to be most closely related in

<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Insect</th>
<th>GenBank accession</th>
<th>Sequence identity (%)</th>
<th>Reference</th>
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<tr>
<td>PinSP1</td>
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<td>AF356842</td>
<td>70</td>
<td>Zheng et al., 2000</td>
</tr>
<tr>
<td>SliSP1</td>
<td>Spodoptera litura</td>
<td>CAB55603</td>
<td>70</td>
<td>Palli et al., 1998</td>
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<td>AAC35428</td>
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<td>Palli et al., 1998</td>
</tr>
<tr>
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<td>Jones et al., 1993</td>
</tr>
<tr>
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<td>66</td>
<td>Burmester et al., 1998</td>
</tr>
<tr>
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<td>Mi et al., 1998</td>
</tr>
<tr>
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<td>70</td>
<td>Corpuz et al., 1993</td>
</tr>
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<td>44</td>
<td>Wang et al., 1993</td>
</tr>
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<td>AAC35429</td>
<td>61</td>
<td>Palli et al., 1998</td>
</tr>
</tbody>
</table>

* For the C-terminal 258 residues only.
amino acid sequence (70% identity) to both a methionine-rich storage protein from the common cutworm, *Spodoptera litura* (Zheng et al., 2000), and a diapause-associated storage protein from the spruce budworm, *Choristoneura fumiferana* (Palli et al., 1998) (Table 1). Other highly similar LSPs included a basic juvenile hormone-suppressible protein precursor from the cabbage looper, *Trichoplusia ni* (Jones et al., 1993) with 69% sequence identity; a methionine-rich hexamerin precursor from the cecropia moth, *Hyalophora cecropia*.  

Fig. 3. Predicted amino acid sequence of LSP's PinSP1 and PinSP2 from *P. interpunctella* and alignment with 12 other insect LSP sequences. 

Water-soluble LSPs from other lepidopterans include the basic juvenile hormone suppressible protein, TniSP1, from *T. ni* (GenBank accession Q06342), the moderately methionine-rich storage protein from *S. litura*, SliSP1 (GenBank accession CAB55603), the storage protein-2 from *H. cunea*, HcuSP2 (GenBank accession AAD39550), the methionine-rich storage protein SP1A from *M. sexta*, MseSP1 (GenBank accession 228382), the methionine-rich hexamerin precursor from *H. cecropia*, HceSP1 (GenBank Accession AAB86647), the diapause-associated protein 1 from *C. fumiferana*, CfuSP1 (GenBank accession AAC35428), and the deduced larval storage protein from *P. interpunctella*, PinSP1 (GenBank accession AF356842), and PinSP2 (GenBank accession AF356843). LSP signature sequences are boxed. Identical residues shared among 13 LSPs (excluding the partial sequence of MseSP1) are indicated by /H at the bottom of the sequences. Identical residues shared at the C-termini shared among all 14 LSPs are indicated with # at the bottom of the sequences. Hyphens represent sequence alignment gaps.
(Burmester et al., 1998) with 66% sequence identity; and a methionine-rich storage protein from the fall webworm moth, *Hypbantria cunea* (Mi et al., 1998) with 66% sequence identity. PinSP1 was also highly homologous with a partial sequence of methionine-rich storage protein SP1A (MseSP1 in the current nomenclature) from the tobacco hornworm, *Manduca sexta* (Corpuz et al., 1993) at the C-terminal 258 residues with 70% identity.

Surprisingly, within the same species, PinSP2 shared only 44% sequence identity with PinSP1 (Table 1). The former protein was more similar to a methionine-rich storage protein from the tobacco hornworm, *M. sexta* (Wang et al., 1993) with 65% sequence identity; a methionine-rich hexamerin precursor from the cecropia moth, *H. cecropia* (Burmester et al., 1998) with 63% sequence identity; a methionine-rich storage protein from the fall webworm moth, *H. cunea* (Mi et al., 1998) with 62% sequence identity; a basic juvenile hormone-suppressible protein precursor from the cabbage looper, *T. ni* (Jones et al., 1993) with 60% sequence identity; a second methionine-rich storage protein from *S. litura* (Zheng et al., 2000) with 62% identity; and a second diapause-associated storage protein from *C. fumiferana* (Palli et al., 1998) with 61% identity.

Multiple amino acid sequence alignments of PinSP1 and PinSP2 with twelve other insect LSPs indicated that these proteins were identical at 160 amino acids (20% identity, Fig. 3 with partial sequence for MseSP1 not shown).
included in this comparison). The two LSP signature sequences were highly conserved among all of these proteins (boxed sequences in Fig. 3). Phylogenetic analysis indicated that PinSP1 and PinSP2 were rooted in two separate groups (Fig. 4; Burmester, 2001). Members within the PinSP1 and PinSP2 subgroups shared 69±1% and 62±1% amino acid sequence identity, respectively, whereas the identity between two storage proteins from the same species ranged only from 41–45% (mean=43±1%), indicating that the two groups, while derived from the same common ancestor, diverged a relatively long time ago.

3.4. Larval storage protein mRNA expression levels

By using quantitative RT–PCR, expression levels of PinSP1 and PinSP2 mRNAs were determined in second, third, and fourth larval instars, as well as in pupal and adult stages of P. interpunctella. To quantify PinSP1 mRNAs, primers JHSP13F and JHSP2R3 (Fig. 1) were used to simultaneously amplify a 1048-bp cDNA fragment from the target cDNA and an 800-bp fragment from an internal standard, designed by cutting out with Bgl II a 248 bp fragment from the same cDNA. PCR reactions containing a series of dilutions (from low to...
Fig. 4. Phylogenetic relationship between deduced LSP sequences from *P. interpunctella* and twelve homologous insect LSP sequences. Sequence names and the GenBank accession numbers are the same as denoted in Fig. 3. PAM = accepted point mutations per 100 residues.

Fig. 5. (A) Quantitative PCR analyses of two LSP mRNAs (pg/µg of total RNA) at various developmental stages of *P. interpunctella*. (B) Quantitative PCR analyses of two LSP mRNAs (pg/µg of total RNA) in males and females of *P. interpunctella* in the pupal and adult stages.

4. Discussion

In the present study, the nucleotide sequences of cDNAs encoding two putative LSPs of *P. interpunctella* were determined. Based on sequence alignments, these proteins are related to a number of other insect proteins called hexamerins that accumulate to extraordinarily high concentrations in hemolymph and fat body of larval stages. Most of these apparently act as storage proteins that serve as sources of amino acids during non-feeding periods of development, but some have been proposed to play other roles in cuticle formation, hormone or metabolite transport, and immune defense (Kanost et al., 1990; Telfer and Kunkel, 1991; Haunerland, 1996; Burmester, 1999). PinSPs are most similar in size, amino acid composition and sequence to the so-called methion-
ine-rich storage proteins that have been characterized in several other lepidopterans (Ryan et al., 1985; Corpuz et al., 1993; Wang et al., 1993; Pan and Telfer, 1996; Mi et al., 1998). Two juvenile hormone-suppressible hexamerins in *T. ni* also belong to this group of proteins (Jones et al., 1990; Beintema et al., 1994). The physiological functions of these and most other storage proteins in insect growth and development, including the Indian-meal moth, are not well understood.

Based on phylogenetic analysis, it appears reasonable to propose PinSP1 and PinSP2 are rooted in two separate groups whose members within each subgroup share substantial amino acid sequence identity, whereas the identity between two storage proteins from the same species is somewhat less. Apparently, the two groups, although derived from a common ancestor, diverged a very long time ago in evolution (~400 million years ago; Burmester, 2001). All of these proteins apparently exhibit a common hexameric subunit structure. The functional significance of both the subunit structure and the presence of two distinct types of LSPs is unknown. It is interesting to note that in each subgroup, the PinSP proteins have the least similarity and form the outermost branch, indicating an earlier divergence from the other LSP genes.

Transcripts for PinSPs accumulated to their highest levels in both males and females just prior to the larval–pupal transformation with PinSP1 mRNA levels being slightly higher than those of PinSP2. It is unclear how increased mRNA levels relate to associated protein levels in *P. interpunctella*. However, stabilization of the mRNA and/or an increased rate of transcription are the two most likely reasons for the elevated levels of the cytochrome P450 oxidase mRNA and protein in the pyrithroid-resistant house fly strain (Porter and Coon, 1991). Liu and Scott (1998) further proved that increased mRNA transcription is the underlying mechanism for increased oxidase activity in the house fly. In some species, methionine-rich LSPs are more abundant in the female than the male during the last larval instar (Ryan et al., 1985; Corpuz et al., 1993; Pan and Telfer, 1996).

We did not investigate sex differences in mRNA levels during that stage of *P. interpunctella*, but no significant differences occurred during the pupal and adult stages. Expression of methionine-rich hexamerin genes in *T. ni* and *M. sexta* is stimulated by ecdysteroid and suppressed or inhibited by juvenile hormone (Jones et al., 1993; Corpuz et al., 1993). The former hormone also promotes the uptake of methionine-rich proteins by the fat body in the latter part of the last larval instar of *Bombyx mori* and *M. sexta* (Tojo et al., 1981; Webb and Riddiford, 1988a,b). The hormonal regulation of expression and uptake of *P. interpunctella* storage proteins has not yet been investigated. The regulation of expression of two other types of hexamerin genes, a riboflavin-binding hexamerin and an arylphorin, in another pyralid species, the waxmoth *Galleria mellonella*, also has been shown to be under hormonal control (Memmel and Kumaran, 1988; Memmel et al., 1994).

Finally, with regard to the amino acid storage function of these proteins, it would appear that the *Plodia* proteins are not just methionine rich in composition, but they also are enriched in aromatic amino acids as well. On the average, proteins contain <2% methionine and only about 8% aromatic amino acids (Heinrikson and Kramer, 1974). PinSP proteins, however, contain >4% methionine and >10% aromatic amino acids. Thus, these proteins would be able to supply a good balance of both of these amino acids to the pupal and adult stages of this species.

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**References**


