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Full Length Research Paper

Prokaryotic expression of vitellogenin receptor gene of *Actias selene* Hubner

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Vitellogenin receptor plays a key role in the embryonic development of oviparous animals. A vitellogenin receptor (VgR) gene was cloned from *Actias selene* using reverse transcriptase-polymerase chain reaction and rapid amplification of cDNA end PCR. Sequence analysis revealed that this gene was 5848 bp and encoded a protein of 1812 amino acids peptide with high similarity to *Bombyx mori* VgR. Sodium dodecyl sulfate polyacrylamide gel electrophoresis and western blotting analysis demonstrated that a 24 and 40 KD recombinant proteins corresponding to the ligand-binding domains of *A. selene* VgR was successfully expressed in *Escherichia coli* cells.

Key words: *Actias selene* Hubner, vitellogenin receptor (VgR), prokaryotic expression.

INTRODUCTION

In insects, vitellogenin receptor (VgR) plays an important role in ovary maturation by mediating the uptake of vitellogenin (Wileman et al., 1985; Raikhel and Dhadialla, 1992; Tufail and Takeda, 2009). Up to now, the VgRs have been identified from many insect species such as *Aedes aegypti* (Sappington and Kokoza,1996), *Blattella germanica* (Ciudad et al., 2006), *Bombyx mori* (Lin et al., 2005), *Drosophila melanogaster* (Schonbaum et al., 1995), *Solenopsis invicta* (Chen et al., 2004), *Periplaneta Americana* (Tufail and Takeda, 2005), *Leucophaea maderae* (Tufail and Takeda, 2007), American dog tick (Mitchell et al., 2007), *Spodoptera litura* (Krishnan et al., 2008) and *Antheraea pernyi* (Liu et al., 2011). All these VgRs belong to the low-density lipoprotein receptor (LDLR) super family (Willnow, 1999) and have common structural elements like the transmembrane domain, ligand-binding domain, the epidermal growth factor precursor domain and cytoplasmic tail (Goldstein et al., 1985; Schneider et al., 1999). However, there is a difference in their physiological roles (Schneider et al., 1999; Nykjaer and Willnow, 2002; Strickland et al., 2002; Herz and Bock, 2002).

Actias selene (Lepidoptera, Saturniidae) is an important wild silk-spinning insect located in China, Japan, India and Southeast Asian countries. Although the vitellogenin was identified from this insect (Qian et al., 2011), the exact biological functions of VgR in *A. selene* remain unknown. In this study, we reported the sequence of *Ash*-VgR, the prokaryotic expression and protein purification of ligand-binding domains of *Ash*-VgR.

MATERIALS AND METHODS

The experimental insect *A. selene* Hubner was collected from the willows in Dangtu, Anhui Province, China.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from the fat body of larvae with TRIzol™ Reagent (Invitrogen) according to the manufacture's instructions. The RevertAid™ H Minus First Strand cDNA Synthesis Kit was used to synthesize cDNAs for reverse transcriptase-polymerase chain reaction (RT-PCR). For rapid amplification of cDNA end (RACE-PCR), the cDNA was synthesized using SMART™ RACE cDNA Amplification Kit (Clontech) according to the manufacturer's instructions.

Cloning and sequencing of VgR

Oligonucleotide primers (Table 1) were designed by Primer premier 5.0 software according to VgR sequences from *B. mori* and *A. pernyi*. RT-PCR was performed at 94°C for 5 min, followed by 30

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Table 1. The primers used for PCR.

Primer No.	Primer sequence (5'--3')
F1(564-585)	TTGCCTCCCGAGTGAAATGATG
R1(2321-2341)	TCCGGGGACCTCTGATGTTGA
F2(1708-1730)	CACCCGTGCTCCCATTTCTGTCT
R2(3301-3320)	CGGTCGCGGCATTGATAGTA
F3(2955-2978)	CGGGCTCTGCGTGGCTAAGGATTC
R3(4491-4513)	AGGCGGACCAGACCACGGACTCG
F4(4454-4474)	CGTCACGGCGCATGTTCTACT
R4(5163-5182)	CTTCGTCGGAGCTGTTCTGG
LBD1ClassAF	GGCGGATCCGAATGCCTGGGCGAG
LBD1ClassAR	CGCCTCGAGGTTGGCACAGAAGGA
LBD2ClassAF	CAGAAGCTTTGAGATGCAGCGAAAGCG
LBD2ClassAR	CGCCTCGAGCGAGAAGCAGTGGATGC

cycles of 94°C for 30 s, 55°C for 40 s, 72°C for 1 min and a final step of 72°C for 10 min. The PCR products were analyzed on 1% agarose gels, then subcloned into the pMD19-T easy cloning vector (Takara) and sequenced at Invitrogen, Shanghai.

Construction of recombinant plasmids and protein expression

Total RNA from the fat body was reverse transcribed into cDNA by a First-Strand System Kit (MBI) according to the protocol. The primers (Table 1) were designed to amplify the cDNA encoding ligand-binding domains (LBD1Class A = 85~642 and LBD2ClassA = 2788~3827) of Ash-VgR protein and PCR products vector were ligated with pET-28a after digesting with corresponding restriction enzymes (*Bam* HI and *Xho*I for LBD1, *Hind* III and *Xho*I for LBD2). The recombinant plasmids pET-28a-Ash-LBD1 and pET-28a-Ash-LBD2 were confirmed by PCR and sequencing, then transformed into *E. coli* BL21 (DE3) for protein expression induced by 0 to 1.0 mM IPTG.

Protein purification and antibody preparation

Ni–NTA (nickel–nitrilotriacetic acid) affinity chromatography (Qiagen) was used to purify the recombinant Ash-LBD1 and Ash-LBD2 proteins according to the instructions. The New Zealand White rabbits were immunized with 100 μg of purified proteins thrice at 2-week intervals and a boost injection was given for another week with purified proteins. Antiserum was collected seven days after the last immunization (Harlow and Lane, 1999)

Western blotting

The recombinant proteins from *E. coli* BL21 (DE3) were subjected to 12% SDS-PAGE and then transferred onto a polyvinylidene difluoride membrane by an electrophoretic transfer system (Bio-Rad). Membranes were blocked with 5% non-fat milk powder (diluted with phosphate-buffered saline containing 0.1% Tween 20 (PBST) for 1 h at room temperature. Membranes were washed with PBST and subsequently incubated with primary antibodies (diluted 1:2000 with PBST) for 2 h at room temperature. After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated sheep anti-rabbit IgG antibody for 1 h at room temperature (Zhu and Wu, 2008), and the final detection was performed with a HRP-DAB Detection Kit (Tiangen).

RESULTS

Cloning and sequence analysis of Ash-VgR gene

A cDNA fragment of 5848 bp was obtained by RT-PCR and RACE–PCR. The sequence had been deposited in the GenBank database with accession number JQ809472. Nucleotide sequence analysis revealed that VgR cDNA contained a 138 bp 5'-untranslated sequence, a putative ORF of 5439 bp and a 271 bp 3′-untranslated region (3′UTR). Based on the deduced amino acid sequences, the LDL-receptor class A domains (residues 29 to 213 and residues 930 to 1278), EGF-like domains (residues 260 to 296 and residues 1316 to 1354), and LDL-receptor class B repeats (residues 344 to 854 and residues 1530 to 1574) were found using the ExPASy Proteomics tools (Figure 1). Phylogenetic analysis indicated that *A. selene* VgR gene was highly homologous to *B. mori* VgR (Figure 2).

Protein expression and western blotting

To investigate the function of *Ash*-VgR, two ligand-binding domains (LBD1 and LBD2) were selected for protein expression. Two recombinant proteins with a molecular weight of about 24 and 40 kDa were detected by SDS-PAGE and their expression was not influenced by different IPTG concentrations (Figure 3a and b). The result of western blotting showed that two consensus 24 and 40 kDa protein bands were detected using anti-His antibodies (Figure 4a and b) or *Ash*-LBD1 and *Ash*-LBD2 antibodies (Figure 5a and b), while there was none in the control group. All these indicated the recombinant VgR proteins were successfully expressed in *E. coli* BL21 (DE3) cells.

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-138                                                        GGGGTACTTTTCCCAAAG
-120 GGGTAAAAATTTAAATTTTTAAATTTAAACCCTTTTTTTTTTGTAATACATCAATCATTT
-60  TAATTCATATTTACCTTCACAATCGTCGATGA AGAATCGCCCTCAACACAATAGATCAGA
1    ATGAAGGTAGTTTTGTTAGCAATAGTTCTATGCGCAACCTCGTGCGCGGGGCAGTTCGT
1    M K V V L L A I V L C A T S C A G Q F V
     signal peptide
61   GACGAAATGCAAGTCTACGAGAAGGAATGCCTGGGCGAGGATGTGTTTCCGTGCATGTCC
21   D E M Q V Y E K E C L G E D V F P C M S
121  GGGGGATGCATACAGCAGTCCCAGTACTGCGACGGGAAGGTGGACTGCGACGATGGAACC
41   G G C I Q Q S Q Y C D G K V D C D D G T
181  GACGAGAACTATTGTCTTGATCACAAGCCAGACGCTCAGTTCTGTAACGAGACCCACCAG
61   D E N Y C L D H K P D A Q F C N E T H Q
241  TTCATGTGTCGGGATAGCAAGAAGTGCATCCCGAACCATTGGATCTGTAATAACGACATC
81   F M C R D S K K C I P N H W I C N N D I
301  GATTGCGACGACGGAAGTGATGAGCTAAATTGCACTTTGGTTCCTGTGGCTACTGGTAAA
101  D C D D G S D E L N C T L V P V A T G K
361  TGCAAAGGTTTTCTGTGCGGCGATGGAAAATGTATCTCCAGTCTTTGGTTATGTGATGGA
121  C K G F L C G D G K C I S S L W L C D G
421  AGCTACGACTGCAAGGATAAGAGCGATGAGAATTCACCGGAAAACTGCCGTCACAGCCTC
141  S Y D C K D K S D E N S P E N C R H S L
481  CTGTCCCACTCGATGCTAAGCGGATCGGATTGCCAGGATTGGCTAGGAGGGAGGCGCCAA
161  L S H S M L S G S D C Q D W L G G R R Q
541  TACAAATGCACGGACTCCTCGTTTTGCCTCCCGAGTGAAATGATGTGTGATGGCATGCAG
181  Y K C T D S S F C L P S E M M C D G M Q
601  GACTGCAAGGACGGCAGTGACGAGAGATCCTTCTGTGCCAACTGGCACACGATGTGCGCG
201  D C K D G S D E R S F C A N W H T M C A
661  AACCACACGTGCCTCGGTGACAAGGCCTCGTGTGTGCCGGACCGCGCCGGGCCCACGTGC
221  N H T C L G D K A S C V P D R A G P T C
721  GAGTGTCTCAACCACCTCAACCTGCGTCGGTACAATACCTCGACCGGGGCCTGCGACGAC
241  E C L N H L N L R R Y N T S T G A C D D
781  ATCGACGAGTGCGCGCTGGCCCGCCCTCAGTGCTCCCACTACTGCGTCAACGCGGACGGC
261  I D E C A L A R P Q C S H Y C V N A D G
841  CATTTCACTTGTGAATGCGCCGACGGCTACTTCAAGGACGAACTTAAGTACTTGTGCTAC
281  H F T C E C A D G Y F K D E L K Y L C Y
901  GCTACCGGTCCCGAACCCCTGTTGTTCTACAGTACACGAAACGAAATTAAATATCTGAAA
301  A T G P E P L L F Y S T R N E I K Y L K
961  GTGAAGTCGAAGGAAGTGGTCACACTGGCGACTGGAATAAAAAAGGCTCACGGGGTCACA
321  V K S K E V V T L A T G I K K A H G V T
1021 TCGAACGGAATATACGTTTACTGGGTGGAAACAGCTGAAGGTCATCAAGCCATCGTCAAA
341  S N G I Y V Y W V E T A E G H Q A I V K
1081 GCTCACATAGACGACGTAGAAAACACTCGACAGGTAATAGTCGGTCTAGGTCTAGAGGAT
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Figure 1. Nucleotide sequence and amino acid sequence of VgR from *Actias selene* Hubner. Start codon (ATG) and termination codon (TAA) are boxed. Signal peptide is underlined, EGF-precursor domain is indicated by wave lines, cytopiasmic domain is indicated by broken lines, ligand-binding domain is Coarse underlined. O-Linked sugar domain is indicated by point line. Transmembrane domain is indicated by long dash point.

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361  A H I D D V E N T R Q V I V G L G L E D
1141 CCAGGCGATATAGCCATTGATTTCATGGCCCGCCACATTTACTTCGGCGATGCTGAAAGG
381  P G D I A I D F M A R H I Y F G D A E R
1201 GGCCTGATCTTCGTATGCTACGATAGCGGCTTCAAATGTTTTACTTTGAAAGCTGACACC
401  G L I F V C Y D S G F K C F T L K A D T
1261 AAACATCCCAAGTTCATCACTCTGGACCCGGTGCACGGGAAGATGTACTGGGCCGATTGG
421  K H P K F I T L D P V H G K M Y W A D W
1321 CACAGCCGGCCGGTGATAATGAGGGCCAAGATGGACGGGTCGAGCTCTGAGGTGCTGGTA
441  H S R P V I M R A K M D G S S S E V L V
1381 GAGTCGATGACGTCATTCGCCAGTGGCCTGGCGCTGGACGTGCCCAACGACAGACTCTAC
461  E S M T S F A S G L A L D V P N D R L Y
1441 TTTGTTGATAAGACCATCAAAGTTGTTCTTCTAAGCACTAAAGTAGTTTACTCGTTGTTC
481  F V D K T I K V V L L S T K V V Y S L F
1501 AAAGAGGCCCACCACCATCCTTACGCGATATCGGTGTTCGAGAACACGGTGTACTGGAGC
501  K E A H H H P Y A I S V F E N T V Y W S
1561 GATTGGATATCAGACTCCATCCAGACTACAGATAAGATTCACAGCTCTTCGCAGAGACAG
521  D W I S D S I Q T T D K I H S S S Q R Q
1621 GTGCTGCTCAAGATGGACACTTCGGTATTTGGTCTCCATATGTACCACCCAGCGTTGATG
541  V L L K M D T S V F G L H M Y H P A L M
1681 AAGAAGATTCCTCATCCGTGCGACGAGCACCCGTGCTCCCATTTCTGTCTGGTCACATCA
561  K K I P H P C D E H P C S H F C L V T S
1741 ATCGACACCTACTCGTGTGCTTGTCCAGACGAAATGGAAAACAAGAACGGCAGATGCATC
581  I D T Y S C A C P D E M E N K N G R C I
1801 CCCAAAGATGACTATCGCCCTCTGCATCTGATAGTCGGCAGCGGTAGACTGTTCACCAAG
601  P K D D Y R P L H L I V G S G R L F T K
1861 TTCCGGTTGGACGCCATGGGCAATCCGCACAGTCACGTCACCAACTTCTCCTTGGGACGC
621  F R L D A M G N P H S H V T N F S L G R
1921 GTGCAAGCTATGACCTATGACTCTGTTCGAGATAGGCTGTATGTGTACGACGGTCGAGAG
641  V Q A M T Y D S V R D R L Y V Y D G R E
1981 CACTCGATCAGCTATACGAACATGAGCGATTTCACTCACGGCAAAGTGTTCGCCCTGATC
661  H S I S Y T N M S D F T H G K V F A L I
2041 AAGTTCGGACCCGAGAACGTTGTGGATATGGACTACGATTACGTCTCGGACTCTCTGTAC
681  K F G P E N V V D M D Y D Y V S D S L Y
2101 ATGCTGGACTCTGGCAGCGGCTACATTGAGGTGTTGTCCTTGCGTACGCTACATCGCGCC
701  M L D S G S G Y I E V L S L R T L H R A
2161 GTCGTCTACCGCTTCACCGACCGGGAGACTCCCGTCAGCTTCTGCGTGCTGCCGCATTAC
721  V V Y R F T D R E T P V S F C V L P H Y
2221 GGGAAAATGTTGGTAGCGGTGATGCAGACGGATAACGACAACCGGATTTATGTGGACAGC
741  G K M L V A V M Q T D N D N R I Y V D S
2281 ATCGGCTTGGATGGAGACGGGAGGCGGCACATCGTCACCGTCAACATCAGAGGTCCCCGG
761  I G L D G D G R R H I V T V N I R G P R
2341 ATAATCCTGAGGTTCTTGCACGGCATGGACAATGTGTACCTGGCGGACGAGGGAAACGGC
781  I I L R F L H G M D N V Y L A D E G N G
2401 ATCATAGATTACCTGCACCCTGAAGGTACCGGTAGGGAGAACTTCCGGGAGCTATCGACT
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Figure 1. Contd.

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 801 I I D Y L H P E G T G R E N F R E L S T
2461 TCAATATCCAGTATGGCCGTCACCGAAAACTATATATTCTGGACAGATAGAAGAACCCCG
 821 S I S S M A V T E N Y I F W T D R R T P
2521 AAGCTATACTGGGCTAATATACACGAAACCTCTCATAAAATCAGAAGGATCGAACTTAGG
 841 K L Y W A N I H E T S H K I R R I E L R
2581 GCATTCTCAAACTCCTCTCAGCTCCTGCTGCAGACCACGTACCCCCCACCGTCTCCTCAC
 861 A F S N S S Q L L L Q T T Y P P P S P H
2641 GACCCGCTCACCCAGCATCCGTGCCACAGAGACAACCCGTGCTCCCAGGTCTGCGTCCCG
 881 D P L T Q H P C H R D N P C S Q V C V P
2701 ACCTATTCCCCCACCAACCCCTACAGCTATAAATGCCTATGCTCTCCGGGTCTCGTGTTC
 901 T Y S P T N P Y S Y K C L C S P G L V F
2761 AGTAACGGGAGATGCACGGAGGTGGCCAGATGCAGCGAAAGCGAAATTTATTGTCACAAA
 921 S N G R C T E V A R C S E S E I Y C H K
2821 AGCAATATATGCGTGGAGAAATTCAAGAGGTGCTGTGGAGTCGTGGATTGCTCGAGGGGG
 941 S N I C V E K F K R C C G V V D C S R G
2881 GAGGACGAAGAAGGATGTACACATATTACAAAGAAGCCAGAAAGCCAGTGCGACCCCAAT
 961 E D E E G C T H I T K K P E S Q C D P N
2941 GAGATACTCTGCTACGGGCTCTGCGTGGCTAAGGATTCCCCTTCCCCTTGTTCGCCTGGG
 981 E I L C Y G L C V A K D S P S P C S P G
3001 AAACATTCAGCTGTTGCAGACCTGACGACCCTTCCCCCTCTGAAATGCGACTGGAACCAG
1001 K H S A V A D L T T L P P L K C D W N Q
3061 TTCACGTGCAAGGAGAGCCCGGTCTGCATCTCGCGGTCGCTGCTCTGTGACGGAGCCAAG
1021 F T C K E S P V C I S R S L L C D G A K
3121 GACTGTCCGGACGGCAGCGACGAGGGCCCCGACAACTGTGACACCTTGGCTTGCTTTGAC
1041 D C P D G S D E G P D N C D T L A C F D
3181 ACGGAGTTCATGTGCGCGTCCGGTTCGTGTATCTTGAAAACGTGGAAGTGCGACGGAGAC
1061 T E F M C A S G S C I L K T W K C D G D
3241 CAGGACTGCAACGACGCTTCCGATGAAATCGACTGTGAGAGCGTATCATGCAAGCCCGGG
1081 Q D C N D A S D E I D C E S V S C K P G
3301 TACTATCAATGCCGCGACCGGGAGTGTATAGAGCTGAAGAAGCGCTGCGACGGACACCAG
1101 Y Y Q C R D R E C I E L K K R C D G H Q
3361 GACTGCTTTGATTACTCCGACGAGGAAGAGTGTGATGAGCCAGTGGCCGTGGAGGAGCCG
1121 D C F D Y S D E E E C D E P V A V E E P
3421 AAAATACATCGTTGTGCCGAATGGGAGTACAGTTGCGAGCGTAACAGAAGTATCTGTTTA
1141 K I H R C A E W E Y S C E R N R S I C L
3481 CCGATTACGGCAAGGTGCAACATGAAAACCGACTGCCCTGGTGGAACGGATGAGATAGGC
1161 P I T A R C N M K T D C P G G T D E I G
3541 TGCGACTACCGGTGCACTCCTCACGGCATGTTCGGTTGCAAGCAGCAGATCCGGTGCTTG
1181 C D Y R C T P H G M F G C K Q Q I R C L
3601 GCCATGAACCGGGTTTGCGACGGAAACAAGGAGTGCGACGATGGATCTGATGAGACGCCC
1201 A M N R V C D G N K E C D D G S D E T P
3661 GACGCTTGCGCTCTCGTCAACAGAACCTCCCACCTGTACCCGGTGATGCTGTATCCGGCA
1221 D A C A L V N R T S H L Y P V M L Y P A
3721 GCAGAGTGCCGCGACGGATTCCTCTGCGGCAACGGTCAATGCATCGAGTGGGCGGAAGTG
```

Figure 1. Contd.

1241 A E C R D G F L C G N G Q C I E W A E V

3781 TGCGACCGCACCCCCAACTGCTTCGACGGATCGGACGAGAGCATCCACTGCTTCTCGGCG

1261 C D R T P N C F D G S D E S I H C F S A

3841 TGCGACAACAACACGTGCGCCCACGCGTGCCAGGCCACGCCGCTGGGGCCGCGCTGCCTG

1281 C D N N T C A H A C Q A T P L G P R C L

3901 TGTCCGGCCGGGTACAGCGCCGCGCCGGACCGCCGGACGTGCGCCGACGTGGACGAGTGC

1301 C P A G Y S A A P D R R T C A D V D E C

3961 CGCGCGGGACTGTGCTCGCAGGCCTGCGTCAACACCCCCGGCTCCTTCCTCTGCTCGTGC

1321 R A G L C S Q A C V N T P G S F L C S C

4021 CATCACGGGTACGCGCTTAGGTCCGACAGACGGTCGTGCAAGGCCGTCACCGGGAACATG

1341 H H G Y A L R S D R R S C K A V T G N M

4081 TCCATACTGTACGTGTCTGGCAACACCGTGCGGTCCGTCTCGGCTGACGGCTACGGCGCT

1361 S I L Y V S G N T V R S V S A D G Y G A

4141 ATAGAGTATAGCGACCCGGACCTTGGCGATATCACAGATTTGGACTTTAATGTCAGAACG

1381 I E Y S D P D L G D I T D L D F N V R T

4201 AAGCGTTTGTATGTGACGTCTACGGAGTCGGGGAAGCTGATAGAATTGAACGTGACGCAT

1401 K R L Y V T S T E S G K L I E L N V T H

4261 GACGTGGTCGCCGTGACGAACGTCGGACGGCCGACCAGGGTGGCAGTGGACTGGGTGACG

1421 D V V A V T N V G R P T R V A V D W V T

4321 GGCAACGTGTACTTCGCGGACAGCACGCCGGGTGCTAGCTGCGTGAGGGTCTGTGACGTC

1441 G N V Y F A D S T P G A S C V R V C D V

4381 ACCAGGAGGAGATGCGCCAGGCTGCAGAAGATACCGTCTGACGCAACGGTCAAGGCATTG

1461 T R R R C A R L Q K I P S D A T V K A L

4441 ATAGTGGAGCCGGCGTCACGGCGCATGTTCTACTGCGTCCAGCGCGGCCACGAGTCCGTG

1481 I V E P A S R R M F Y C V Q R G H E S V

4501 GTCTGGTCCGCCTCGCTCTCAGGCCGGAGCGCCCTGGACCTCCTCCACGTGACCCAGTGC

1501 V W S A S L S G R S A L D L L H V T Q C

4561 TCGGGTTTAGCTGCCGATTCGTTCACGAGGAGGCTGTATGTGGCAGAGACTGCGCCCCCC

1521 S G L A A D S F T R R L Y V A E T A P P

4621 CACATCATGGTCGTCGACTTCGATGGCAAGAATCCCAAGAAGATCCTGACGGAACGTCCA

1541 H I M V V D F D G K N P K K I L T E R P

4681 CAGCTGCAAGCGCCCCACGCCTTGGCGCTCTTCGAAGACCACATATACTATTTGGTGGGC

1561 Q L Q A P H A L A L F E D H I Y Y L V G

4741 GACTCGTACCGCCTCGGGCGCTGCCTGCTCCACGGCCCTAAGAACTGCGAGACCTACATC

1581 D S Y R L G R C L L H G P K N C E T Y I

4801 TACAGGGTGTTCGAAGCGAACACCTTCGTCATCAGACACGAGAGCATCCAGCGCGACGAC

1601 Y R V F E A N T F V I R H E S I Q R D D

4861 CTGGTCAACGAGTGCGCCGGCCACGACTGCTCCAATGTGTGCGTGCTCGAGAAGGCTCCG

1621 L V N E C A G H D C S N V C V L E K A P

4921 GTGTGTGTCTGCGACGACGGGCACGTCCGTGACGACGGGAACTGTGACCCCAGCAGCAAA

1641 V C V C D D G H V R D D G N C D P S S K

4981 AACGAGCTCCCCCTGTTCAACGGCTGGACGTACCAGGACTATCAGCGCGGTCACCGCGCC

1661 N E L P L F N G W T Y Q D Y Q R G H R A

5041 AGCATCACCGTCGTCATCGCGGTCCTCGTGCTGTTCCTCGTGTACATAGCACTGTTTGTA

Figure 1. Contd.

1681 S I T V V I A V L V L F L V Y I A L F V

5101 TATTATCACTTCGTCTATAAACCAAAGAGGAAGAGGTCCACGGCTTATACAGAGGTGAGG

1701 Y Y H F V Y K P K R K R S T A Y T E V R

5161 TTCCAGAACAGCTCCGACGAAGCAGCGCAGTTGTCTTGCAGCCCGGCAGTCCAAATGAAT

1721 F Q N S S D E A A Q L S C S P A V Q M N

5221 GGAAATCAACTTATCAATGGTAACGAATTCGTGAACCCGCTCCAGTACGTGCGCAACGTG

1741 G N Q **L** **I** N G N E F V N **P** **L** **Q** **Y** V R N V

5281 TGGCAACAATCTATCAGAAGGAAGCCACGTCCTGTTTGTACAGCTGGCCTGTCAATAGCA

1761 W Q Q S I R R K P R P V C T A G L S I A

5341 GTGCCTAACTCTCCACAGCAAGACTTCTCCGATACAGAGTCAGATCTAGACGATCGAGAA

1781 V P N S P Q Q D F S D T E S D L D D R E

5401 ACAAAGAGGTTTATCCTCAAAAATAAGTTTCTCAAT$\boxed{\text{TAA}}$CTTAAGTTACAGGAAATGTCG

1801 T K R F I L K N K F L N

5461 TTAAATTTTTTTGCTGAGCAAAAAATGGATGGCATATTCCGAATTTTATATTTTAATCCT

5521 ACTATTTAAGATTTAGATTAGGTTATGATATAGCATAACTAACTTCTAGCTTGTTAAATT

5581 ATTTTATTGTTTGAATGTTATCAAAATAGTTTTTATTTGCTAAATTTTATACATAAAATG

5641 TATCGATATTGTATTGATTGAATTTTGAAAATAAAACATTGATTTATATGAGAAAAAAAA

5701 AAAAAAAAAA

Figure 1. Contd.

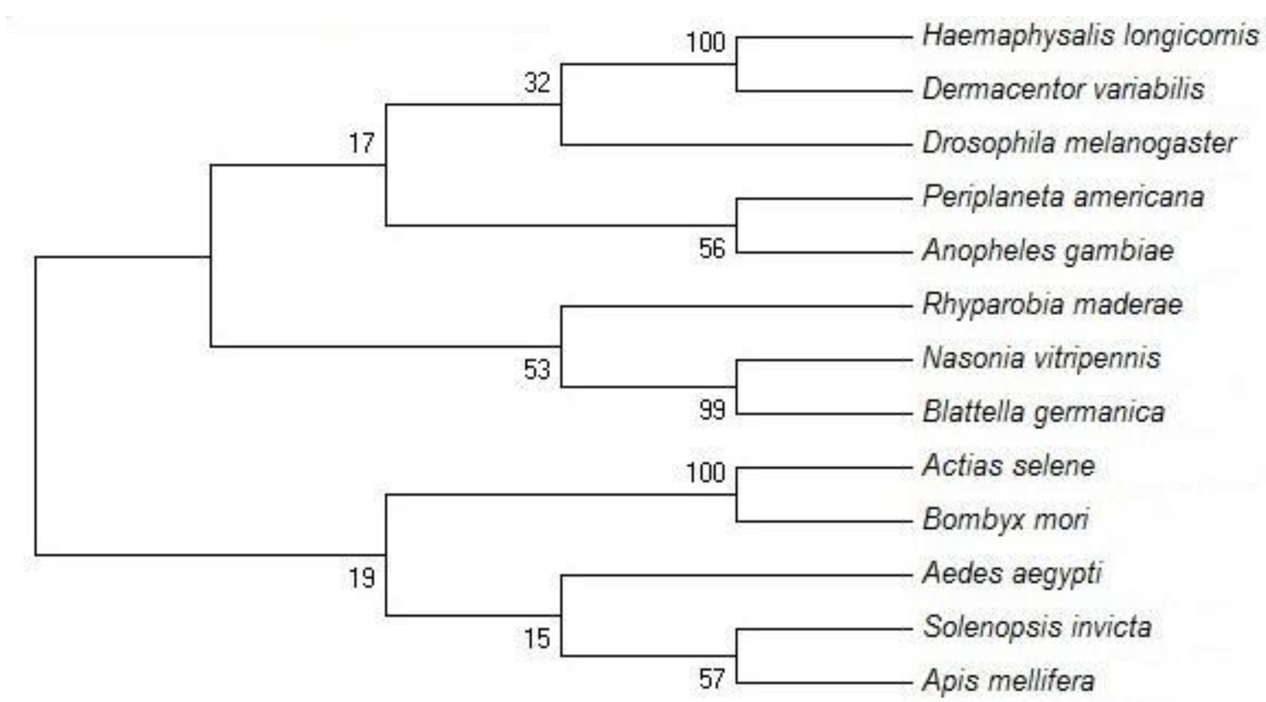

Figure 2. Phylogenetic analysis was performed by MEGA (version 4.0) program based on the VgRs from various insects. The phylogenetic tree was constructed using the neighbor joining algorithm method and bootstrap values (1000 repetitions) of the branches are indicated. The *VgR* proteins are from: *Bombyx mori* (HM172611), *Drosophila melanogaster* (U13637), *Aedes aegypti* (L77800), *Anopheles gambiae* (EAA06264), *Solenopsis invicta* (AY262832), *Nasonia vitripennis* (XM-001602904), *Apis mellifera* (XM-001121707), *Periplaneta americana*(AB077047), *Blattella germanica* (AM050637), *Rhyparobia maderae* (AB255883), *Dermacentor variabilis* (DQ103506.4), *Haemaphysalis longicornis* (AB299015).

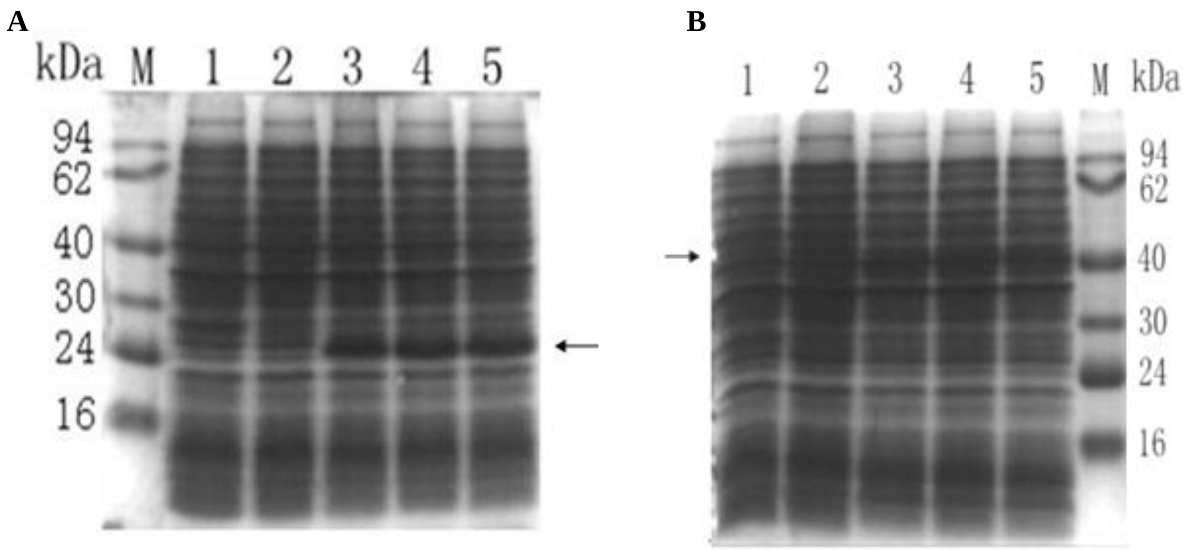

Figure 3. Analysis of recombinant pET-LBD1 (a) and pET-LBD2 (b) proteins on 12% SDS-PAGE gels. The gels were revealed by Coomassie blue R-250 staining. M, protein marker; lane 1, pET-28a; lane 2, without induction; lanes 3 to 6, after induction by 0.3, 0.6, 1.0 mM IPTG, respectively.

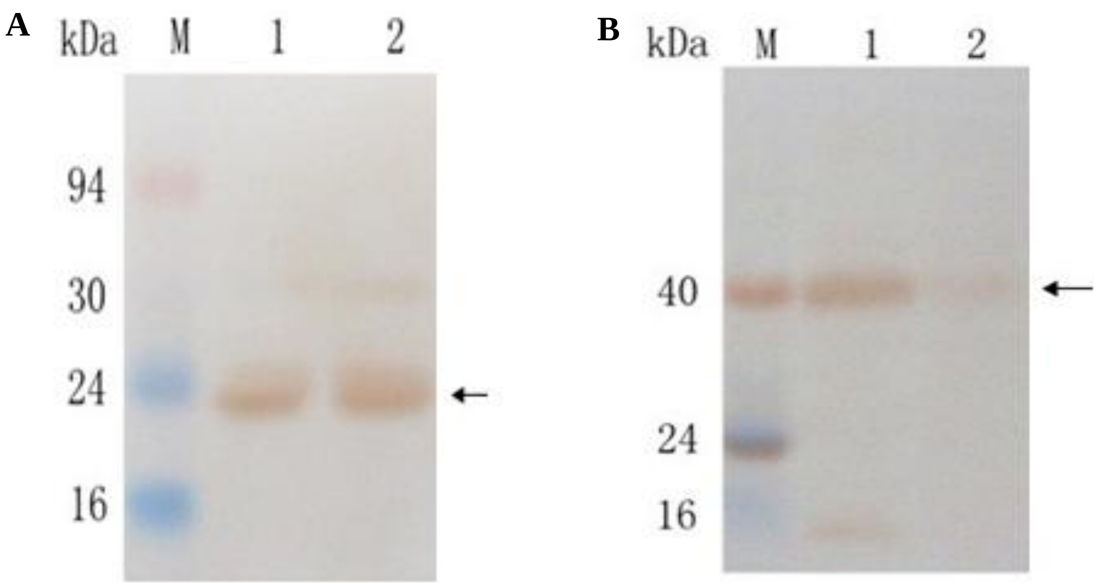

Figure 4. Western blot analysis of recombinant proteins with anti His-tag antibody. A 24 kDa (a) and a 40 kDa (b) protein band were detected by western blotting. No immunoreactive band was found in the control group. M, protein marker. Lanes1-2, after induction by 0.3, 0.6 mM IPTG, respectively; Lane 3, No IPTG induction.

DISCUSSION

In this study, a full-length cDNA encoding VgR gene was identified from *A. selene* and the predicted protein consists of 1812 amino acids with a calculated molecular mass of 203.9 kDa, which is similar to other insect VgRs (Sappington and Kokoza, 1996; Liu et al., 2011). Sequence analysis shows *Ash*-VgR is highly homologous to *B. mori* and has the conserved structures as found in other insects (Tufail and Takeda, 2009).There are eleven cysteine-rich repeats in *Ash*-VgR like *Ap*-VgR reported in our previous study (Liu et al., 2011), however, this structure is different from vertebrate VgRs (Tufail and Takeda, 2009). In addition, the prokaryotic expression and purification of *Ash*-VgR were successfully performed in this experiment, and the interaction between *Ap*-Vg and *Ap*-VgR will be further carried out to investigate the biological functions of *Ash* –VgR.

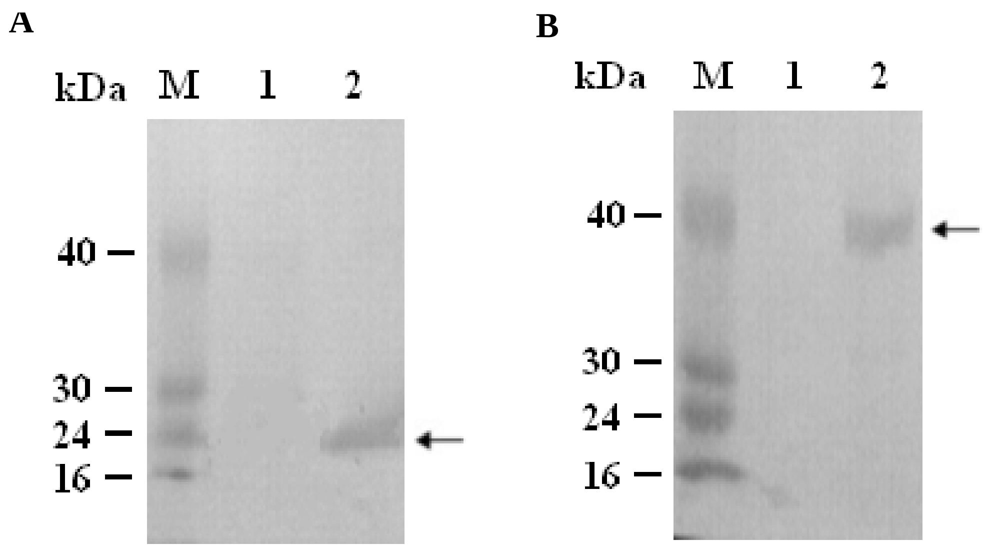

Figure 5. Western blot analysis of recombinant proteins using antigen specific antibodies. (a) Western blotting of pET-LBD1, (b) western blotting of pET-LBD2. Lane 1, without induction; lane 2, after induction.

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REFERENCES

Chen ME, Lewis DK, Keeley LL, Pietrantonio PV (2004). cDNA cloning and transcriptional regulation of the vitellogenin receptor from the fire ant, *Solenopsis invicta Buren* (Hymenoptera: Formicidae). Insect Mol. Biol., 13: 195–204.

Ciudad L, Piulachs MD, Belles X (2006). Systemic RNAi of the cockroach vitellogenin receptor results in a phenotype similar to that of the Drosophila yolkless mutant. FEBS. J., 273: 325–335.

Goldstein JL, Brown MS, Anderson RGW, Russell DW, Schneider WJ (1985). Receptor-mediated endocytosis: concepts emerging from the LDL receptor system. Annu. Rev. Cell. Biol., 1: 1-39.

Harlow E, Lane D (1999). Using antibodies: a laboratory manual. Cold Spring Harbor Laboratory Press.

Herz J, Bock H (2002). Lipoprotein receptors in the nervous system. Ann. Rev. Biochem., 71: 405-434.

Krishnan M, Muthumeenakshi P, Bharathiraja C, Warrier S, Subramoniam T (2008). A comparative study on vitellogenin receptor of a lepidopteran insect (*Spodoptera litura)* and a decapod crustacean (*Scylla serrata*): Phylogenetic implication and co-evolution with vitellogenins. J. Endocrinol. Reprod.*,* 1:13-23.

Lin Y, Zhao P, Hou Y, Li J, Gong DP, Su Q, Xia QY, Xiang ZH (2005). Vitellogenin and vitellogenin receptor gene of the silkworm *Bombyx mori*. Acta. Zoologica. Sinica., 51: 117-125.

Liu QN, Zhu BJ, Liu CL, Wei GQ, Wang ZG (2011). Characterization of vitellogenin receptor (VgR) from the Chinese oak silkworm, Antheraea pernyi. Bulletin. Insectol., 64: 167-174.

Mitchell RD, Ross E, Osgood C, Sonenshine DE, Donohue KV, Khalil SM, Thompson DM, Roe RM (2007). Molecular characterization, tissue-specific expression and RNAi knockdown of the first vitellogenin receptor from a tick. Insect Biochem. Mol. Biol., 37:375–388.

Nykjaer A, Willnow TE (2002). The low-density lipoprotein receptor gene family: a cellular Swiss army knife? Trends. Cell. Biol. 12:273-280.

Qian C, Liu CL, Zhu BJ, Meng Y, Wei GQ (2011). Identification and expression analysis of vitellogenin from silk-producing insect, *Actias selene* Hubner. Afr.J. Biotechnol., 10: 999-1010.

Raikhel AS, Dhadialla TS (1992). Accumulation of yolk proteins in insect oocytes. Annu.Rev. Entomol., 37: 217-251.

Sappington TW, Kokoza VA (1996). Molecular characterization of the mosquito vitellogenin receptor reveals unexpected high homology to the Drosophila yolk protein receptor. Proc. Nat. Acad. Sci., 93:8934-8 999.

Schneider WJ, Nimpf J, Brandes C, Drexler M (1999). The low-density lipoprotein receptor family, genetics, function, and evolution. Curr. Atheroscler. Reports., 1: 115–122.

Schonbaum CP, Lee S, Mahowald AP (1995).The Drosophila yolkless gene encodes a vitellogenin receptor belonging to the low density lipoprotein receptor superfamily. Proc. Nat. Acad. Sci. 92:1485–1489.

Strickland DK, Gonias SL, Argraves WS (2002). Diverse roles for the LDL receptor family. Trends. Endocrinol. Metabol., 13: 66-74.

Tamura K, Dudley J, Nei M, (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24: 1596-1599.

Tufail M, Takeda, M (2005). Molecular cloning, characterization and regulation of the cockroach vitellogenin receptor during oogenesis. Insect Mol. Biol., 14: 389–401.

Tufail M, Takeda M (2007). Molecular cloning and developmental expression pattern of the vitellogenin receptor from the cockroach, *Leucophaea maderae*. Insect Mol. Biol., 37: 235–245.

Tufail M, Takeda, M (2009). Insect vitellogenin/lipophorin receptors: Molecular structures, role in oogenesis,and regulatory mechanisms. J. Insect. Physiol., 55:88–104.

Wileman T, Harding C, Stahl P (1985). Receptor-mediated endocytosis. Biochem. J., 232: 1-14.

Willnow TE (1999). The low-density lipoprotein receptor gene family: multiple roles in lipid metabolism. J. Mol. Med., 77: 306-315.

Zhu BJ, Wu XZ (2008). Identification of outer membrane protein *ompR* from rickettsia-like organism and induction of immune response in *Crassoatrea ariakensis*. Mol. Immunol., 45: 3198-3204.