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Full Length Research Paper

Identification and expression analysis of vitellogenin from silk-producing insect, *Actias selene* Hubner

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This study mainly shows the DNA sequence and expression analysis of vitellogenin in *Actias selene (Ash*-Vg*).* Specific primers were designed to amplify *Ash*-Vg gene by polymerase chain reaction (PCR) and the obtained DNA sequence was 7329 bp long, including 6 exons and 5 introns with an open reading frame encoding a 1774 amino acids peptide. A Bm DSX binding site and some conserved signatures such as CdxA and GATA-X were found in the 5′-flanking region of *Ash*-Vg gene. Meanwhile, the cDNA encoding the small subunit of *Ash*-Vg protein was obtained by PCR and ligated to pET-28a vector for protein expression. A 45 KD recombinant protein was successfully expressed in *Escherichia coli* cells which were confirmed by sodium dodesyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis. The semi-quantitative PCR was also carried out to explore the specific expression of *Ash*-Vg and the results showed that the *Ash*-Vg gene expressed differently in various developmental stages and tissues.

Key words: *Actias selene* Hubner, vitellogenin, DNA sequence, expression.

INTRODUCTION

Vitellogenin (Vg), the precursor of major yolk protein in insects (Wahli et al., 1981; Kunkel and Nordin, 1985; Sappington et al., 2002), is synthesized as one or more large precursors (Della-Cioppa and Engelmann, 1987) in fat body and secreted into the hemolymph. It will then be up taken by vitellogenin receptors (VgRs) through receptor-mediated endocytosis (Raikhel and Dhadialla, 1992; Sappington and Raikhel, 1998; Snigirevskaya and Raikhel, 2005). Vgs played important roles in promoting growth and differentiation of oocytes and transporting metallic ions, lipid, thyroxine, vitamin A, carotenoid and riboflavin into oocyte. So far, Vgs have been sequenced from 25 insect species. Alignment of the Vgs' sequences revealed that some conserved features such as cysteine residues, the GL/ICG and DGXR motifs, were found (Tufail and Takeda, 2008) at the carboxy terminal.

Among the Lepidoptera insects, the Vgs have been identified from *Bombyx mori* (Yano et al.,1994a; Yano et al.,1994b), *Bombyx mandarina* (Meng et al., 2006), *Antheraea pernyi* (Yokoyama et al.,1993; Liu et al., 2001, Zhu et al., 2010), *Antheraea yamamai* (Liu et al., 2000; Meng and Liu, 2006a), *Saturnia japonica* (Meng et al., 2008), *Samia cynthia ricini* (Kajiura et al., 2008), *Lymantria dispar* (Hiremath and Lehtoma, 1997) and *Philosamia cynthia ricini* (Liu et al., 2003). Researches on insect Vgs provide great theoretical and practical significances for utilization of beneficial insects and prevention of harmful insects (Brownes, 1986). The primary structures of many insect Vgs (Tufail and Takeda, 2008) and some advanced structure are known (Sharrock et al., 1992).

Actias selene Hubner (Lepidoptera, *Saturniidae*) is an important wild silk-spinning insect mainly located in China, Japan, India and Southeast Asian countries. Vg of

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Abbreviations: Vg, Vitellogenin; ***Ash*-Vg,** vitellogenin from *Actias selene* Hubner; **VgR,** vitellogenin receptor; **Aa,** amino acid; **bp,** base pairs; **LB,** Luria-Bertani; **IPTG,** isopropylthiogalactoside; **X-gal,** 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside; **PCR,** polymerase chain reaction; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Table 1. The primers used for polymerase chain reaction (PCR).

Primer	Primer sequences
F1	5′- TGTAATAACAGTCGATCTATCCATGTAG -3′
R1(6-26)	5′- ACCACCGCTAGAACCAACAAC -3′
F2(1-22)	5′- ATGAAGTTGTTGGTTCTAGCGG -3′
R2(1040-1059)	5′- ACGACGTGATCTCTGCTTCG -3′
F3(446-466)	5′- ATCGTAACATCCATGGCTCTC -3′
R3(1462-1481)	5′- TCGCGATACACCATCCACAT -3′
F4(1288-1307)	5′- CTGCAAGATATTGCTCAGCA -3′
R4(2182-2202)	5′- TGCTGATTTAAGAGCTGAGC -3′
F5(2061-2080)	5′- TACCGCAGAACCCTATGAAG -3′
R5(2674-2693)	5′- TCACTGATATCGTTAGGCAG -3′
F6(2594-2611)	5′- ATCCTCTTGAAGCCTCCT -3′
R6(3366-3385)	5′- GATACCTGAGGTTAAAGTGC -3′
F7(3259-3280)	5′- GGATATTCGTATTCAACAGATT -3
R7(3604-3623)	5′- AGTACGTAATCAGCCTTATG -3′
F8(3487-3506)	5′- GCAGACTTTAGTCCGAACAG -3′
R8(4252-4271)	5′- TGTGGCTGGAACGGCTGATA -3′
F9(4143-4162)	5′- GGCTTCATACTTCGACCAGA -3′
R9(4674-4693)	5′- CTTCGATGTTCAGCATCAGG -3′
F10(4540-4557)	5′- CCTGAAGGCAGCAAACAA -3′
R10(5351-5370)	5′- TTTTCCGTTTTCAATGCCTA -3′

A. selene Hubner (Ash-Vg) has two subunits (175 and 45KD) as observed through sodium dodesyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting performed by Dong et al. (2003). However, the exact biological functions of Vgs in *A. selene* Hubner remain unknown. In this study, we reported the DNA sequence of *Ash*-Vg and the prokaryotic expression of *Ash*-Vg, and the *Ash*-Vg specific expression in different developmental stages. Tissues were also detected by semi-quantitative polymerase chain reaction (PCR).

MATERIALS AND METHODS

Materials

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

Extraction of genomic DNA and total RNA

Fat body was collected from female pupa, washed with distilled water and then grounded quickly with liquid nitrogen. Phenol-chloroform method was used (Mahendran et al., 2006) for the extraction of genomic DNA. Total RNA was extracted using TRIzol™ Reagent (Invitrogen) according to the instructions. The extracted DNA and RNA were stored at -70°C.

Cloning of *Ash*-Vg

Oligonucleotide primers (Table 1) were designed to amplify the genomic DNA sequence of *Ash*-Vg according to its cDNA sequence (Yin et al., 2007). PCR was performed according to the procedure as follows: 5 min at 94°C; followed by 34 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min and a final step of 72°C for 5 min. The PCR products were analyzed on 1% agarose gels, then purified with DNA gel extraction kit (AxyGen) and ligated with pMD19-T easy cloning vector (Takara), then transformed into *Escherichia coli* (DH5α). The positive clone was selected by PCR and sequenced at Invitrogen, Shanghai.

Construction of recombinant expression plasmid and protein expression

Total RNA from fat body was reverse transcribed into cDNA by a First-Strand System Kit (Promega) according to its manufacturer's protocol. The primers EpF1: 5′-CGCGGATCCATGAAGTTGTTGGTTCTAGCGG-3′ and EpR1: 5′-CCCAAGCTTCTAACGACGTGATCTCTGCTTCG-3′ (restriction enzyme sites BamHI and HindIII were underlined) were designed to amplify the cDNA encoding small subunit (1-353 aa) of *Ash*-Vg protein and PCR products vector were ligated with pET-28a after digesting with restriction enzymes (BamHI and HindIII). The recombinant plasmids pET-28a-*Ash*-Vg was transformed into *E. coli* BL21 (DE3) for protein expression induced by 1 mM IPTG.

Western blotting

The recombinant fusion protein after SDS-PAGE was transferred onto a polyvinylidene difluoride membrane by an electrophoretic transfer system. Membranes were blocked with phosphate-buffered saline containing 0.1% Tween20 and subsequently incubated with anti-His tag antibodies for 2 h at room temperature, washed by Phosphate Buffered Saline Tween-20 (PBST) and incubated with horseradish peroxidase-conjugated sheep anti-rabbit IgG antibody

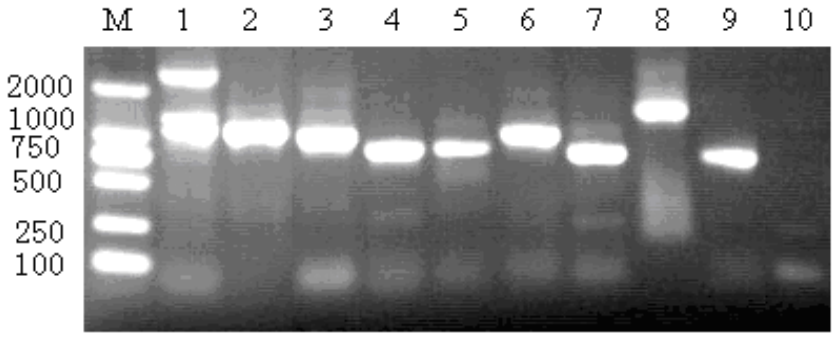

Figure 1. PCR amplification results of *Ash*-Vg DNA. Lanes 1 - 10, PCR products using primer pairs from F_1R_1 to $F_{10}R_{10}$, the products sizes are about 1000, 1000, 900, 800, 800, 1000, 800, 1600, 800 and 250 bp, respectively; M, marker.

(Sigma) for 1 h at room temperature according to the method of Zhu and Wu (2008). The final detection was performed with a HRP-DAB Detection Kit (Tiangen).

Expression of *Ash*-Vg in different developmental stages and tissues

Semi-quantitative PCR was carried out with specific primers *Ash*F1: GGATATTCGTATTCAACAGATT and *Ash*R1: AGTACGTAATCA GCCTTATG to determine the expression level of *Ash*-Vg and 18s rRNA gene was used as an internal reference (with primers 18sF: CGATCCGCCGACGTTACTACA and 18sR: GTCCGGGCCTGGT GAGATTT). The amplification program for semi-quantitative PCR was the same as described above except for 22 cycles of amplification.

RESULTS

Sequence of *Ash*-Vg gene

Primers were designed according to the reported sequence of Vgs from *A. selene* Hubner*, A. pernyi* and *A. yamamai* (Li et al., 2008; Meng and Liu, 2006a) to identify the sequence of *Ash*-Vg gene by using genomic DNA as templates and ten DNA fragments were obtained through PCR amplification (Figure 1). *Ash*-Vg gene is 7329 bp long (GenBank No. GU361974), including a 206bp 5′-flanking region, some conserved signatures such as CdxA, GATA-X, Bm DSX and TATA box, and five introns and six exons were found in this sequence (Figures 2a and b).

Protein expression and western blotting

With a pair of primers, EpF1 /EpR1, we amplified the small subunit of *Ash*-Vg gene from the genome. Through prokaryotic expression of this obtained small subunit, a recombinant protein with a molecular weight of about 45 kDa was shown by SDS-PAGE (Figure 3). Western blot analysis of recombinant protein showed that a desired protein band can be detected in recombinant plasmids PET-*Ash*-Vg induced by isopropylthiogalactoside (IPTG), while none in the control group (Figure 4). All this indicates the successful expression of small subunit of *Ash*-Vg in *E. coli* cells.

Expression of *Ash*-Vg in various developmental stages and tissues

Semi-quantitative PCR was carried out to detect the specific expression of *Ash*-Vg in different developmental stages and tissues with specific primers. Results showed that the expression of *Ash*-Vg was not detected in the whole larva stages, and its expression reached the maximum in prepupa stage but decreased with development (Figure 5a). In addition, *Ash*-Vg was mainly expressed in blood, fat body and ovary tissues (Figure 5b).

DISCUSSION

Comparing the obtained DNA sequence of *Ash*-Vg gene with those of *A. pernyi*, *A. yamamai*, *B. mori*, *L. dispar* and *Abies grandis* showed similarity in their structures while they were obviously different from *Aedes aegypti* and *Anopheles gambiae* for these two species had three exons and two introns (Meng et al., 2006b). However, the first big intron (31 bp followed ATG) existed in insects *B. mori*, *A. yamamai* and *L. dispar* but was absent in the *Ash*-Vg gene. Except for this intron, size and location of other exons were similar while introns changed greatly, which further demonstrated the diversity of vitellogenin gene in insects. Increasing evidence shows that introns were involved in gene expression regulation, especially the first intron which contains DNA replication and transcription regulatory elements (Xie and Wu, 2001). Interestingly, the first big intron is just missing in *A. selene* Hubner*;* whether this intron is related with the function of *Ash*-Vg and the relationship between these introns are

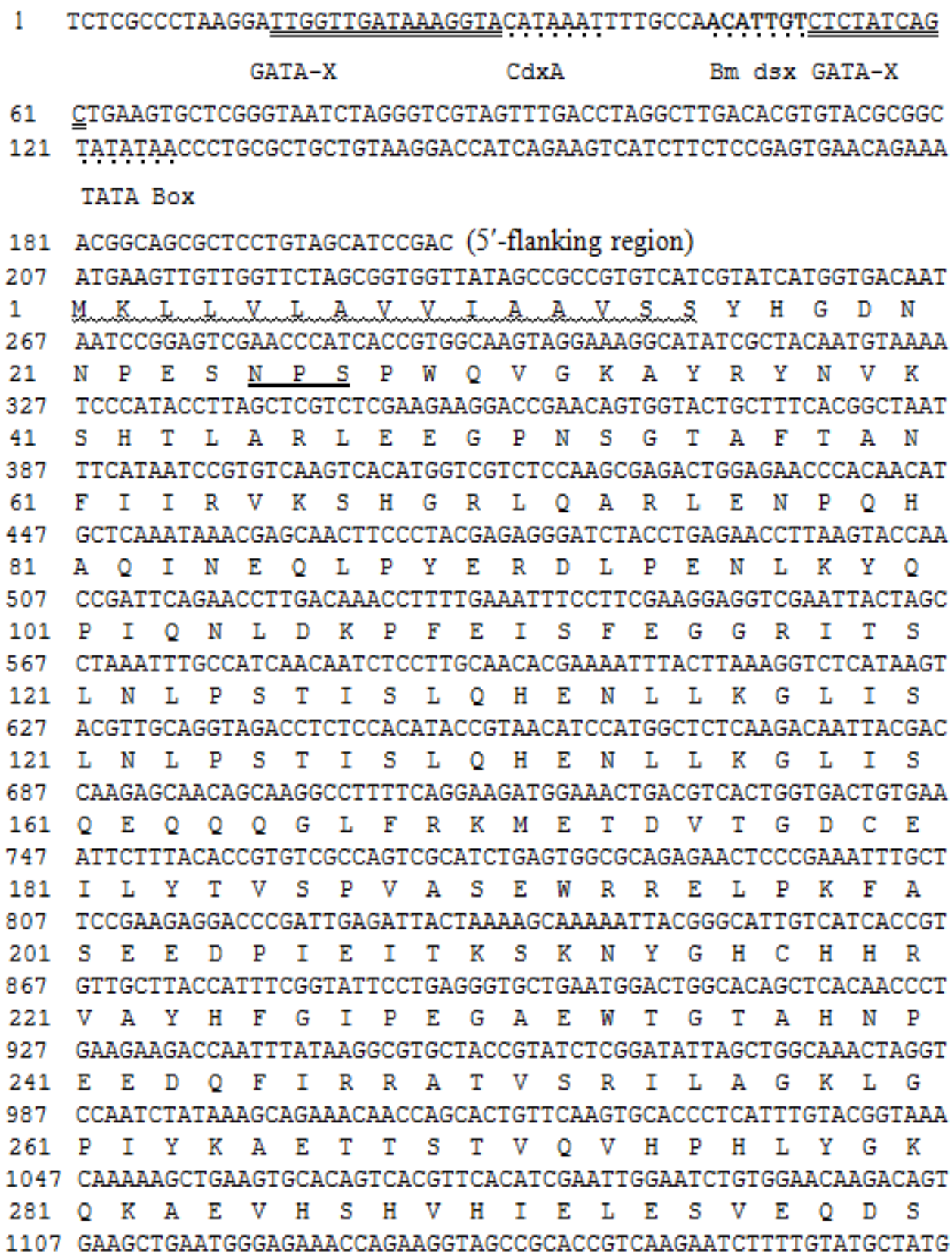

Figure 2a. Nucleotide sequence and amino acid sequence of *Ash*-Vg. Signal peptides is indicated by wave lines, N-linked glycosylation sites are underlined, broken lines represent ploy serine region, protein restriction enzyme cutting site are boxed, sequence with a consensus DGQR and GICG are shaded, introns are indicated by black and italics.

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301  E  A  E  W  E  K  P  E  G  S  R  T  V  K  N  L  L  Y  A  M
1167 TCAACAAAACAGATCGCTACACATGATAGCTCGTCCTCATCGTCTTCGGAGTCACATGAA
321  S  T  K  Q  I  A  T  H  D  S  S  S  S  S  S  S  E  S  H  E
1227 CATGCAATCAATGAGGAACCGAAGCAGAGATCACGTCGTTCTATGAGAGCATCTAAAGTC
341  H  A  I  N  E  E  P  K  Q  R  S  R  R  S  M  R  A  S  K  V
1287 GGTGCTATACAGAATTACATGAGTCAACAAAAGAAGCACAGAGATGATAGTTCGAGTTCA
361  G  A  I  Q  N  Y  M  S  Q  Q  K  K  H  R  D  D  S  S  S  S
1347 TCTTCTTCTAGTTCTAGCTCAGACTCGTCATCTGCCTACATTAACGACGAGATGCCCGGC
381  S  S  S  S  S  S  S  D  S  S  S  A  Y  I  N  D  E  M  P  G
1407 CTTAATGACCCTGTCTACGCTGCACTGTATATGAGTCCTCAAACTCATACTGATAAGAAA
401  L  N  D  P  V  Y  A  A  L  Y  M  S  P  Q  T  H  T  D  K  K
1467 CAAAATTCAGTTAACGCTCAAAAGCTTCTGCAAGATATTGCTCAGCAATTACAGAACCCC
421  Q  N  S  V  N  A  Q  K  L  L  Q  D  I  A  Q  Q  L  Q  N  P
1527 AACAATATGCCAAAATCAGATTTTCTTTCTAAATTTAACATCCTTGTACGTTTAATCGCT
441  N  N  M  P  K  S  D  F  L  S  K  F  N  I  L  V  R  L  I  A
1587 AGCATGTCAACTGAACAATTAAGCCAGACTAGCCGTACTATCGAAGCTGGTAAGTCCTCT
461  S  M  S  T  E  Q  L  S  Q  T  S  R  T  I  E  A  G  K  S  S
1647 AACAACAACATCAAAAAAGATATGTGGATGGTGTATCGCGATGCTGTAACCCAAGCTGGT
481  N  N  N  I  K  K  D  M  W  M  V  Y  R  D  A  V  T  Q  A  G
1707 ACTTTACCTGCTTTCCAACAAATTAAAAGCTGGATTAATTCCAAGAAGATCCAAGATGAA
501  T  L  P  A  F  Q  Q  I  K  S  W  I  N  S  K  K  I  Q  D  E
1767 GAAGCAGCCCAAGTCGTAGCCAGTTTGTCTTCTACTCTGCGCTATCCTACGAAAGAAGTT
521  E  A  A  Q  V  V  A  S  L  S  S  T  L  R  Y  P  T  K  E  V
1827 ATGATACAATTCTTCAAACTTGCGAGAAGCCCTGAAGTGAAAGATCAATTATACCTTAAC
541  M  I  Q  F  F  K  L  A  R  S  P  E  V  K  D  Q  L  Y  L  N
1887 ACCACAGCTCTTATTGCTGCAACCAGGTTCATTAATATGGGTCAAGTGAATAATTACACA
561  T  T  A  L  I  A  A  T  R  F  I  N  M  G  Q  V  N  N  Y  T
1947 GCCCATAACTTCTACCCAACCCATATGTACGGACGACTTGCGCGCAAACACGACAACTTT
581  A  H  N  F  Y  P  T  H  M  Y  G  R  L  A  R  K  H  D  N  F
2007 GTTCTTGAACAGATTTTACCTCCTCTTTCTGAGGACCTGAAAAATGCTATCCAGCAACAA
601  V  L  E  Q  I  L  P  P  L  S  E  D  L  K  N  A  I  Q  Q  Q
2067 GACAGCGTCAAAGCGCAAGTTTATGTAAAGGCTATTGGTAATTTGGGACATCCTGAAATA
621  D  S  V  K  A  Q  V  Y  V  K  A  I  G  N  L  G  H  P  E  I
2127 CTAAAAGTTTTCGCTCCTTATTTAGAAGGTCAAATTAAAGTATCGACTTACCTCCGGGCC
641  L  K  V  F  A  P  Y  L  E  G  Q  I  K  V  S  T  Y  L  R  A
2187 CAAATGGTTTCTAATCTTATTGTTTTGTCCAATCAAAGAAATAAGCAAGCTCGCGCAGTT
661  Q  M  V  S  N  L  I  V  L  S  N  Q  R  N  K  Q  A  R  A  V
2247 CTTTATAGTATTTTGAGGAATACCGCAGAACCCTATGAGGTCAGAGTTGCCGCTATTCAT
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Figure 2a. Continued.

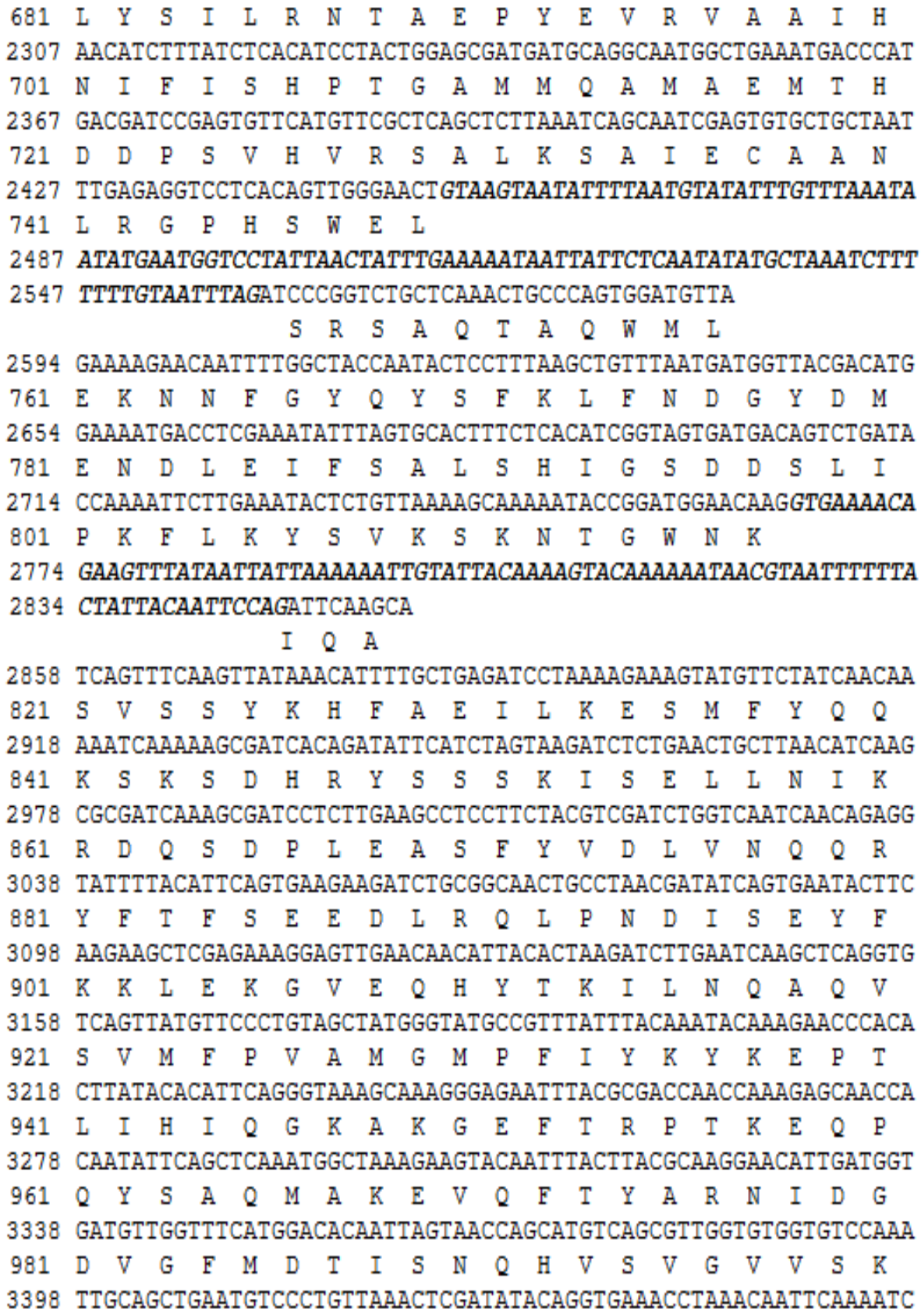

Figure 2a. Continued.

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1021 R  A  E  P  L  H  P  E  Q  D  S  T  I  V  H  Y  S  V  W  P
3518 TACAGTGCTGTTCAAAAGAAGGATTCTATTGTACCAATTTCACTGGACCCCACATCTAAA
1041 Y  S  A  V  Q  K  K  D  S  I  V  P  I  S  L  D  P  T  S  K
3578 GTGGTTGAACGCCAGAGGAAGATTCTGTCGGTTGATACTAAGTTCGGACAAGCTACGAGC
1061 V  V  E  R  Q  R  K  I  L  S  V  D  T  K  F  G  Q  A  T  S
3638 ACCGTCTTCCAATTCCAAGGATATTCGTATTCAACAGATTTCAAGAACTTTGGAACCGTC
1081 T  V  F  Q  F  Q  G  Y  S  Y  S  T  D  F  K  N  F  G  T  V
3698 TTCAACTCGCCAGACTTTATTACAAATATTGCTTCTATATTCTCACAGCAAGACATCGCC
1101 F  N  S  P  D  F  I  T  N  I  A  S  I  F  S  Q  Q  D  I  A
3758 ATGACGCACTTTAACCTCAGGTATCTGGCCAAACAGTCTCAGAATAAGGCTGTTACTTTA
1121 M  T  H  F  N  L  R  Y  L  A  K  Q  S  Q  N  K  A  V  T  L
3818 AGAGCTGTGTACGGTAAGTTATTCCAATGTTTTCTTTTATATTTAAATACCCCACATATA
1141 R  A  V  Y  D
3878 TTGGAAGATAACGAAAAACTATAATTGAAATTTGACATCTACTAGGGTAGCTCCATCTTG
3938 ACTTTCGAGAAATTATTCGCAATTGGTATATCTAAAAAAAACCTATATATTTTTCTTGTA
3998 GGCAATGTTGGCCTAGATTTTAAATAAGCTTTTTCTTTTCATTCTTGTTCTACCTAAGTA
4058 GTATCAACACGACATAGTTTCTTTCATTATATTCTAAATTAAGATTAATCGCCGTACCAT
4118 TCAAACTACCATCGGTTATCATAAATTTCTTGACATCTCATTGCCTATTTAAATATCTTC
4178 GTCTGTTACATAAATATACCGTCACTTTAAAGTGCAACTAAAAATAAAATACTAGTAGTT
4238 AAAATAAACTACATGTATTTTTAATGCTTATAAATTTTTACGATTAAGGGTTTTATACTG
4298 CAAATTTTTTTTGCTACCTTGCTACTCTTGGTACCTACTGTCTTTAAATTGTGTTAGAAT
4358 ATATAATTATGTTATATTACATTTTTGCAACACATTCATTCAAATAAGCCATTATCTTTC
4418 ATATCATTATCATTTCTTATCTACGCACATCATTTTCAGATGATTACTATAATCAAAAAG
                                          D  Y  Y  N  Q  K
4478 AAAGCGGTGAACTGGGTCCGGCCGCT
     E  S  G  E  L  G  P  A  A
4504 GATCAGGCAGACTTTAGTCCGAACAGTGAAGCCCGTCGTCAGGCAATCGCAAAACGTGTT
1161 D  Q  A  D  F  S  P  N  S  E  A  R  R  Q  A  I  A  K  R  V
4564 TCTGCAGGGATCAATACCGCCAAAGCTCAAGTAGTCGACTTCAGCGCTACCTTTGAAGGA
1181 S  A  G  I  N  T  A  K  A  Q  V  V  D  F  S  A  T  F  E  G
4624 AGCCATAAGGCTGATTACGTACTTACAGCCGCCATAAGCGAAAGCCCAGTTGACCCTAAG
1201 S  H  K  A  D  Y  V  L  T  A  A  I  S  E  S  P  V  D  P  K
4684 GTGCAATACGCTCTCTTCGCTGGTAAAAACTCAGCTCAACACGGCAAGAGCCAATTCAAT
1221 V  Q  Y  A  L  F  A  G  K  N  S  A  Q  H  G  K  S  Q  F  N
4744 GCCGTAGGAACAGTAAAATTACCAAGGAGTAATGCCCTGAATTTCTTGCAAGTCCTTGAC
1241 A  V  G  T  V  K  L  P  R  S  N  A  L  N  F  L  Q  V  L  D
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Figure 2a. Continued.

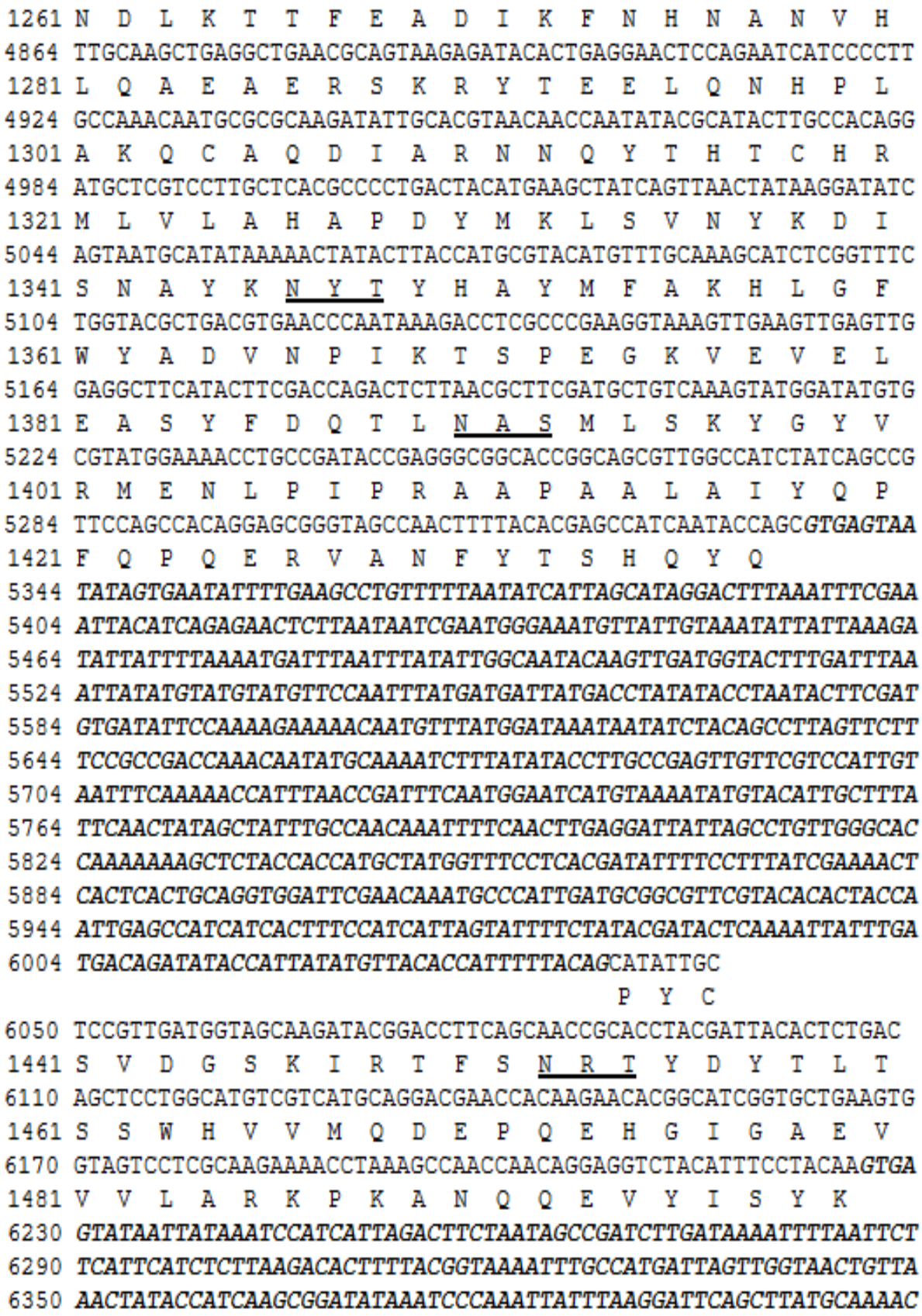

Figure 2a. Continued.

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                                                     S
6460 GAAACTGGCAAAGACCTTGAAATTGAAATTCAACCAGCACCTGAAGGCAGCAAACAACCT
1501 E  T  G  K  D  L  E  I  E  I  Q  P  A  P  E  G  S  K  Q  P
6520 CGAGTTAACGTTAAGACTAATGCAAAGAAGGTGTCTGAAGGTGAATTGACGATTTACTGG
1521 R  V  N  V  K  T  N  A  K  K  V  S  E  G  E  L  T  I  Y  W
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1541 N  D  V  E  Q  K  P  L  L  E  Y  Y  Y  Q  Q  D  G  A  L  M
6640 CTGAACATCGAAGAGTACAAGTTTAGGACAGTCTACGATGGACAGAGGTTGGTTGTATTG
1561 L  N  I  E  E  Y  K  F  R  T  V  Y  D  G  Q  R  L  V  V  L
6700 GCCAGCGAAAACCGTCAGAGTGCTCGCGGTATCTGCGGCAGTATGAGCGGTGAACCTCGT
1581 A  S  E  N  R  Q  S  A  R  G  I  C  G  S  M  S  G  E  P  R
6760 GATGATTATCTGACTCCTGAGGGTTTGGTCGATAAACCCGAACATTACGCCGCTTCGTAC
1601 D  D  Y  L  T  P  E  G  L  V  D  K  P  E  H  Y  A  A  S  Y
6820 GCCCTCAACGATGAGAACAGTGACCCGAGAACCCAGGAACTGAAGGCTAAAGCTAAACAA
1621 A  L  N  D  E  N  S  D  P  R  T  Q  E  L  K  A  K  A  K  Q
6880 GAAGCTTACCAACCTAAGAACAAATATACTACTGTCCTCCGTTCTGATCCGCAATGGCAG
1641 E  A  Y  Q  P  K  N  K  Y  T  T  V  L  R  S  D  P  Q  W  Q
6940 CAACAAATGTCGGCTTCCTCATCATCGGAAGAAGATTGGGGATCCGAAACCGTTTACAGA
1661 Q  Q  M  S  A  S  S  S  S  E  E  D  W  G  S  E  T  V  Y  R
7000 TCGAGGAGCTATGACAAGCAGAGGGGGCCCTGTGCGGTGAAACAACAAGTTCAGTACTAT
1681 S  R  S  Y  D  K  Q  R  G  P  C  A  V  K  Q  Q  V  Q  Y  Y
7060 GAGAACCATGGTGAAATCTGTATCACCACCGAACAGCTGCCAGCTTGCCAGTCGCATTGC
1701 E  N  H  G  E  I  C  I  T  T  E  Q  L  P  A  C  Q  S  H  C
7120 CATGGTGATGAGTACAGGATTCAAGCTGCTCAAGTATCCTGCCGACCCAAGCTTGACCAT
1721 H  G  D  E  Y  R  I  Q  A  A  Q  V  S  C  R  P  K  L  D  H
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1741 Q  Y  R  A  Y  R  D  Q  I  K  Q  G  Q  N  P  T  V  T  G  V
7240 CCCAAGGTAAAACAATTCAAGGTACCAACAGCTTGCAAGGCATAAATAAAGTAGATAAAT
1761 P  K  V  K  Q  F  K  V  P  T  A  C  K  A
7300 GAGAATTAACTAGGCATTGAAAACGGAAAA
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Figure 2a. Continued.

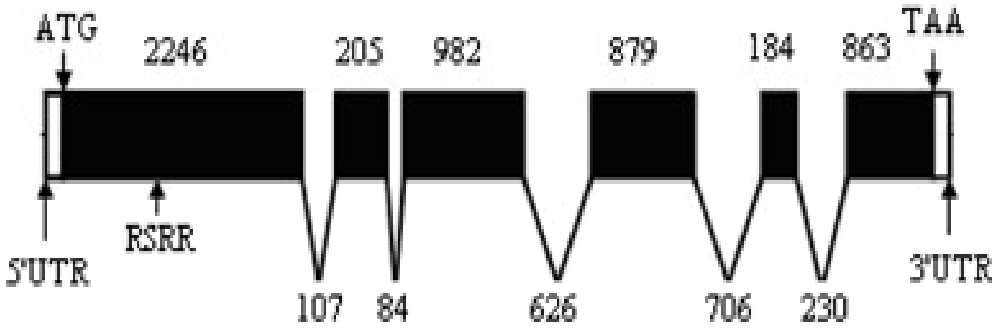

Figure 2b. Structure diagram of the whole *Ash*-Vg gene. Exons are indicated by black, the white gaps between them mean introns. UTRs and RSRR motifs are indicated by an arrow.

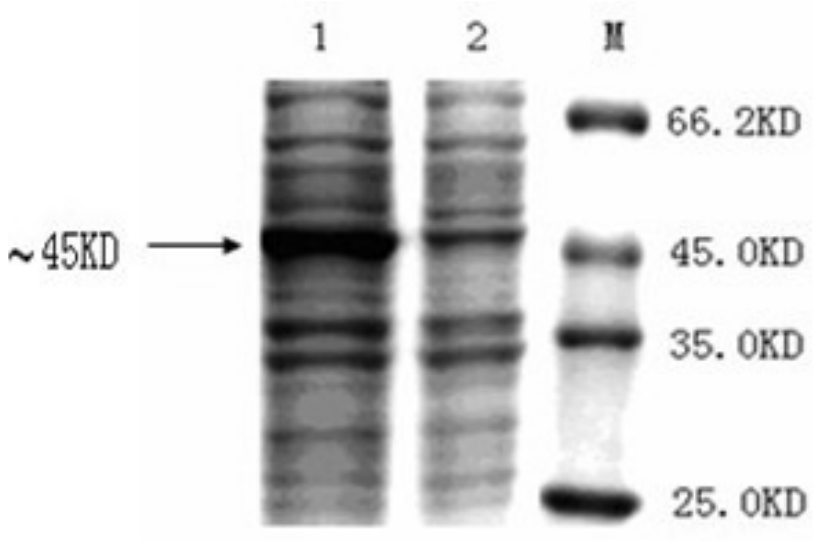

Figure 3. Analysis of recombinant *Ash*-Vg protein on 12% SDS-PAGE gels. The gels were revealed by Coomassie blue R-250 staining. Lane 1, After induction by 1.0 mM IPTG. Lane 2, without induction; M, protein marker.

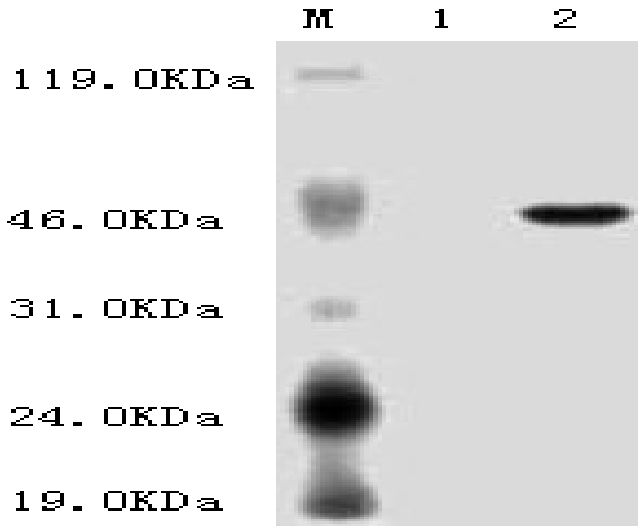

Figure 4. Western blot analysis of recombinant proteins with anti His-tag antibody. A protein band with a molecular mass of about 45 kDa was detected by western blotting. No immunoreactive band was found in the control group. Lane 1, No IPTG induction. Lane 2, after IPTG induction; M, protein marker.

still unknown.

The conserved regulatory elements CdxA and GATA-X in many insect genes were found in 5′ - flanking region of *Ash*-Vg gene as well as the recognition site of Bmdsx (ACATTGT) in the promoter region of the *B. mori* , *A. pernyi* and *A. yamamai* Vg gene (Suzuki et al., 2003; Meng and Liu, 2006a; Li et al., 2008). According to reports, DSX was involved in sex determination cascade and regulated the expression of yolk protein genes and some other sex-specific differentiation genes (Burtis et al., 1991; Jursnich and Burtis, 1993; An and Wensink 1995a, b). At present, little is known about 5′- regulatory region sequence for many insects, so whether DSX gene exist in all other insects still needs further research.

In this study, the expression of *Ash*-Vg reached a high level in prepupa stage and declined in the forth day of pupation which showed the stage-specific characteristics. This may be related to redistribution of protein during organizational reform in metamorphosis period. This is supported by the fact that fat body RNA contents of *A. pernyi*, *A. yamamai* and *Philosamia cynthia ricini* are in a downward trend on the first day of pupation (Liu et al., 2002), and maybe, partial *Ash*-Vg in fat body was secreted into the hemolymph and transported to the developing oocytes (Ye et al., 1997). Oocyte uptake Vg by vitellogenin receptor through receptor-mediated endocytosis. This is the universal mechanism for zooblast to selectively absorb large molecular substances (Lin et al., 2005). In this experiment, small subunit of *Ash*-Vg we successfully expressed which was confirmed by SDS-PAGE and western blotting. This will contribute to the investigation of the relationship between structures and functions of Vgs.

In conclusion, these results provide some useful information for further researches on insect Vgs and its roles in biological procedures and biosynthesis mechanism of Vgs in *A. selene* Hubner.

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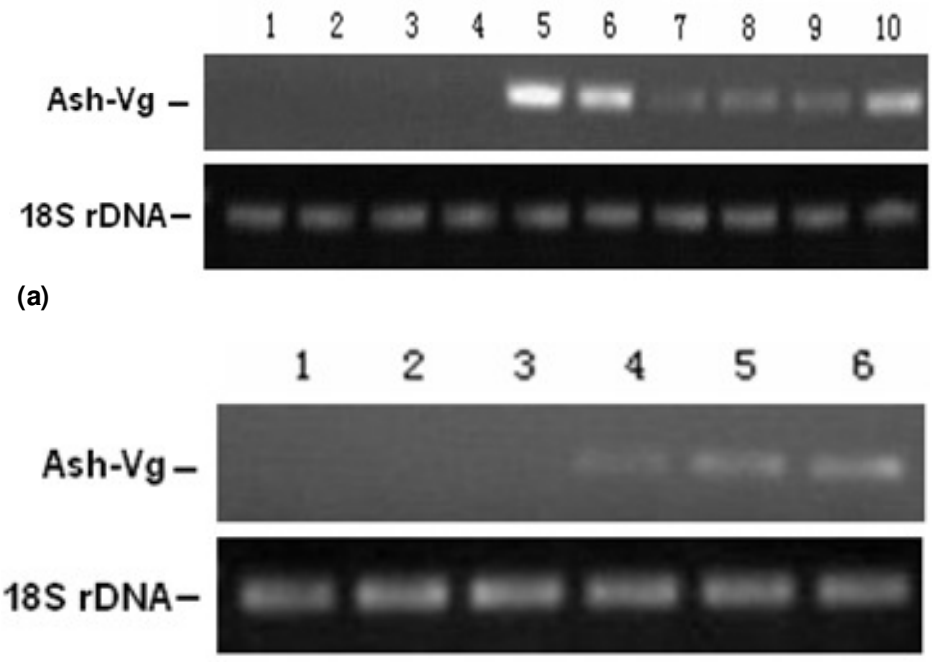

Figure 5. Expression analysis of *Ash*-Vg by semi-quantitative PCR. (a) Specific expression of *Ash*-Vg in different developmental stages. Lanes 1-4, Expression of *Ash*-Vg at the first, fourth, seventh and eleventh day of larva stages, respectively; Lanes 5-10, expression of *Ash*-Vg at the first, forth, seventh and eleventh day of pupae and diapause stages, respectively. (b) Expression of *Ash*-Vg in different developmental tissues. Lanes 1 - 6, Expression of *Ash*-Vg in mid-intestine, head, malpighian tube, blood, fat body and ovary during the fourth day in female pupae, respectively.

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