Int. J. Indust. Entomol. Vol. 7, No. 1, 2003, pp. 51~57

International Journal of *Industrial Entomology*

cDNA Cloning of a Putative Alcohol Dehydrogenase from the Silkworm, *Bombyx mori*

Iksoo Kim, Yong Soo Choi[1], Hung Dae Sohn[1] and Byung Rae Jin[1,*]

Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, Suwon 441-100, Korea.
[1]College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.

(Received 15 May 2003; Accepted 18 July 2003)

A cDNA encoding a putative alcohol dehydrogenase (ADH) class III was cloned from the silkworm, *Bombyx mori*. The full length cDNA is 1,385 nucleotides long and contains an open reading frame of 1,128 bp encoding 376 amino acid residues. The *B. mori* ADH III protein sequence was aligned with ADH III known from various organisms. Interestingly, the protein sequence of *B. mori* ADH III showed 87% and 85% identity to ADH III from marine fish *Sparus aurata* and *Branchiostoma floridae*, respectively, whereas rather low sequence identity (83%) to *Drosophila melanogaster* ADH III was observed. Northern blot analysis revealed that *B. mori* ADH III mRNA is expressed in all tissues from larva examined: fat body, midgut, epidermis, silk gland and ovary, with the highest level found in the fat body.

Key words: Insect, Silkworm, *Bombyx mori*, Alcohol dehydrogenase, ADH, cDNA sequences, mRNA expression

Introduction

Alcohol dehydrogenase (ADH) is the primary enzyme responsible for metabolism of ethanol to acetaldehyde and constitutes a large family of related enzymes and isozymes. Of these ADHs, classes I and III are those best defined. The class I ADH (ADH I, EC 1.1.1.1) is the classic ethanol-active form, evolves rapidly and exhibits a considerable variability between different species (Danielsson *et al.*, 1994). The class III (ADH III, EC 1.2.1.1) is a glutathione-dependent formaldehyde dehydrogenase that can oxidize ethanol at high concentrations (Koivusalo *et al.*, 1989). The ADH III is present in prokaryotes and in all eukaryotes thus far investigated (Danielsson and Jornvall, 1992; Hjelmqvist *et al.*, 1995; Funkenstein and Jakowlew, 1996; Shafqat *et al.*, 1996; Duester *et al.*, 1999; Canestro *et al.*, 2000; Dasmahapatra *et al.*, 2001).

In insect, ADH I is responsible for the catalysis of the reversible conversion of various alcohols generated by microbial fermentation in larval or adult feeding sites to their corresponding aldehydes and ketones (Atrian *et al.*, 1998). Until now ADH gene is mainly investigated in diptean insect species. The ADH genes have been cloned and sequenced from various *Drosophila* species (Batterham *et al.*, 1984; Begun, 1997; Danielsson *et al.*, 1994; Nurminsky *et al.*, 1996). In addition, fundamental molecular differences between the class I and III ADH enzymes are well established in *Drosophila* species (Danielsson *et al*, 1994).

Beside *Droshophila*, genetic information on insect ADH is very limited to a few other species such as the medfly (*Ceratitis capitata*), the olive fruit fly (*Bactrocera oleae*), flesh fly (*Sarcophaga peregrine*) (Benos *et al.*, 2000; Goulielmos *et al.*, 2001; Horio *et al.*, 1996), and mole cricket, *Gryllotalpa orientalis* (Kim *et al.*, 2003a). Thus, all available sources of ADH genes including *Drosophila* are confined to Diptera, except for *G. orientalis*. Biochemical characterization has shown that two ADH proteins exist in the *C. capitata* (Gasperi *et al.*, 1992), encoded by two highly linked genes on the second chromosome, probably generated by gene duplication event (Gasperi *et al.*, 1994; Malacrida *et al.*, 1992). In *B. oleae*, the ADH is a dimmer with molecular weight of 48,000 Da, consisting of two subunits (Mazi *et al.*, 1998) and the two ADH genes, named ADH I and ADH II, consisting of three exons and two introns for a total of 1,981 and 988 nucleotides, respectively, have been reported (Goulielmos *et al.*, 2001). Our research group cloned and

*To whom correspondence should be addressed.
College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea. Tel: +82-51-200-7594; Fax: +82-51-200-7594; E-mail: brjin@mail.donga.ac.kr

sequenced a cDNA encoding an ADH enzyme, which is composed of 798 bp with 266 amino acid residues from *G. orientalis* (Kim *et al.*, 2003a). However, genetic information of the ADH in lepidopteran insects is still unknown.

In this study, we firstly report the cDNA sequence of ADH from lepidopteran insect. We cloned and sequenced a cDNA encoding a putative class III ADH from the Silkworm, *Bombyx mori*. The cDNA sequence and mRNA expression of a putative ADH III from *B. mori* are discussed.

Materials and Methods

cDNA library screening, nucleotide sequencing and data analysis

A cDNA library was constructed using poly (A) + mRNA isolated from the whole bodies of the silkworm, *Bombyx mori*, larvae (Kim *et al.*, 2003b). The cDNA library was screened to generate the expressed sequence tags (ESTs). Sequencing of randomly selected clones harboring cDNA inserts was performed. For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega, Madison, WI). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL, and SwissProt databases were searched for sequence homology using a BLAST algorithm program.

MacVector (ver. 6.5, Oxford Molecular Ltd.) was used to align the amino acid sequence of ADHs. The aligned

Table 1. Alcohol dehydrogenase from various species

Common name	Species	Sequence name	Amino acid size	GenBank accession number	References
Insect					
Silkworm	*Bombyx mori*	*Bm*ADHIII	376		This study
Olive fruit fly	*Bactrocera oleae*	*Bo*ADH1	257	AJ277835	Goulielmos *et al.* (2001)
Olive fruit fly	*Bactrocera oleae*	*Bo*ADH2	258	AJ277834	Goulielmos *et al.* (2001)
Mediterranean fruit fly	*Ceratitis capitata*	*Cc*ADH1	257	Z30194	Benos *et al.* (2000)
Mediterranean fruit fly	*Ceratitis capitata*	*Cc*ADH2	258	Z30195	Benos *et al.* (2000)
Flesh fly	*Sarcophaga peregrina*	*Sp*ADH	257	D63669	Horio *et al.* (1996)
Fruit fly	*Drosophila hydei*	*Dh*ADH1	254	X58694	Menotti-Raymond *et al.* (1991)
Fruit fly	*Drosophila hydei*	*Dh*ADH2	254	X58694	Menotti-Raymond *et al.* (1991)
Fruit fly	*Drosophila buzzatii*	*Db*ADH1	254	U65746	Unpublished
Fruit fly	*Drosophila buzzatii*	*Db*ADH2	254	U65746	Unpublished
Fruit fly	*Drosophila mojavensis*	*Dmoj*ADH1	254	X12536	Bayer *et al.* (1992)
Fruit fly	*Drosophila mojavensis*	*Dmoj*ADH2	254	X12536	Bayer *et al.* (1992)
Fruit fly	*Drosophila montana*	*Dmon*ADH1	254	U26842	Nurminsky *et al.* (1996)
Fruit fly	*Drosophila montana*	*Dmon*ADH2	254	U26845	Nurminsky *et al.* (1996)
Fruit fly	*Drosophila virilis*	*Dv*ADH1	254	U26846	Nurminsky *et al.* (1996)
Fruit fly	*Drosophila virilis*	*Dv*ADH2	254	U26846	Nurminsky *et al.* (1996)
Fruit fly	*Drosophila melanogaster*	*Dmel*ADH	256	AF175220	Begun *et al.* (1999)
Fruit fly	*Drosophila melanogaster*	*Dmel*FDH	380	AY089518	Unpublished
Fruit fly	*Drosophila melanogaster*	*Dmel*ADH	380	U07641	Danielsson *et al.* (1994)
Fish					
Sea squirts	*Ciona intestinalis*	*Ci*ADH3	378	AF344172	Canestro *et al.* (2002)
Florida lancelet	*Branchiostoma floridae*	*Bf*ADH3	378	AF154331	Canestro *et al.* (2002)
Gilthead seabream	*Sparus aurata*	*Sa*ADHIII	377	U84791	Funkenstein *et al.* (1996)
Animal					
Human	*Homo sapiens*	*Hs*ADH5	375	M30471	Sharma *et al.* (1989)
Human	*Homo sapiens*	*Hs*FDH	375	XM_208352	Unpublished
House mouse	*Mus musculus*	*Mm*ADH 2	375	M84147	Hur *et al.* (1992)
Rabbit	*Orytolagus cuniculus*	*Oc*ADH3	375	Y15406	Svensson *et al.* (1998)
Plant					
Thale cress	*Arabidopsis thaliana*	*At*ADHIII	370	AY087250	Haas *et al.* (2002)

amino acid sequences of the GenBank-registered ADH amino acid sequences and putative *B. mori* ADH sequence were subjected to phylogenetic analysis by using PAUP* (Phylogenetic Analysis using Parsimony and Other Method*) ver. 4 (Swofford, 2000). The information and accession numbers of the known insect ADH sequences in the GenBank are described in Table 1.

RNA isolation and Northern blot analysis

B. mori larvae were dissected under the Sterero-microscope (Zeiss, Jena, Germany), individual samples such as fat body, midgut, epidermis, silk gland, and ovary were harvested, and washed twice with PBS buffer. Total RNA was isolated from fat body, midgut, epidermis, silk gland, and ovary of the *B. mori* larvae using the Total RNA Extraction Kit (Promega). Total RNA (10 μg/lane) from the *B. mori* larva was separated on glyoxalation gel (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2×PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the ADH transcripts was 1,385 bp for *B. mori* ADH cDNA cloned in this study and labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2×SSC at 65°C, and finally exposed to autoradiography film.

```
-105                tgcttctggcttatcagaaattcaatacgactcctatagggaat
     tactgttcactgtacggcctactactggattttgtgagattggcagttgtccagtgagtc
   1 ATGTCAACAGTCGGTAAAGTGATTAAGTGCTTGGCCGCAGTCGCGTGGGAAGCAGGCAAG
     M  S  T  V  G  K  V  I  K  C  L  A  A  V  A  W  E  A  G  K
  61 CCGCTGTCCATCGAGGAGATTGAAGTGGACCCGCCAAAAGCCGGTGAAGTGCGCGTTAAG
     P  L  S  I  E  E  I  E  V  D  P  P  K  A  G  E  V  R  V  K
 121 ATCACGGCCACCGGAGTCTGCCATACTGACGCCTATACACTCTCCGGAAAAGATCCTGAG
     I  T  A  T  G  V  C  H  T  D  A  Y  T  L  S  G  K  D  P  E
 181 GGAGTGTTTAATGTAGTACTGGGACATGAAGGCGGCGGAATCGTGGAGAGTGTCGGTGAG
     G  V  F  P  V  V  L  G  H  E  G  G  G  I  V  E  S  V  G  E
 241 GGAGTCACCTCAGTCAAGCCCGGGGACCACGTAGTACCTCTGTACGTCCCACAGTGCAAC
     G  V  T  S  V  K  P  G  D  H  V  V  P  L  V  V  P  Q  C  N
 301 ACATGTAAATTCTGCAAGAATCCGAAAACTAATTTGTGCCAGAAGGTTCGTTCTACTCAA
     T  C  K  F  C  K  N  P  K  T  N  L  C  Q  K  V  R  S  T  Q
 361 GGTCAAGGTGTGATGCCAGATGGCACTAGGCGATTCCGCTGTAAAGGACAGGAACTCTAC
     G  Q  G  V  M  P  D  G  T  R  R  F  R  C  K  G  Q  E  L  Y
 421 CATTTCATGGGTTGTTCAACATTCAGTCAGTACACAGTTGTTCTAGAAATTTCTCTCTGT
     H  F  M  G  C  S  T  F  S  Q  Y  T  V  V  L  E  I  S  L  C
 481 AAAGTTGCAGAGGCGGCTCCATTAGATAAAGTTTGTTTGCTGGGATGCGGTGTACCTACA
     K  V  A  E  A  A  P  L  D  K  V  C  L  L  G  C  G  V  P  T
 541 GGTTATGGAGCCGCCTTGAATACTGCCAAAGTTGAACCAGGATCAAATTGCGCTATTTTT
     G  Y  G  A  A  L  N  T  A  K  V  E  P  G  S  N  C  A  I  F
 601 GGTCTTGGTGCTGTTGGTTTAGCTGTTGCTCTTGGATGCAAAGCGGCAGGTGCCAATCGC
     G  L  G  A  V  G  L  A  V  A  L  G  C  K  A  A  G  A  N  R
 661 ATTATTGGTGTTGACATCAACCCTGACAAGTTTGAGGTAGCTAAGAAATTTGGAGTCAAT
     I  I  G  V  D  I  N  P  D  K  F  E  V  A  K  K  F  G  V  N
 721 GAATTTGTCAACCCTAAGGATTATGATAAACCAATTCAACAAGTATTGGTGGATTTGACT
     E  F  V  N  P  K  D  Y  D  K  P  I  Q  Q  V  L  V  E  L  T
 781 GATGGTGGTCTAGAATACACTTTTGAATGTATTGGAAATGTAGGCACCATGAGAGCTGCA
     D  G  G  L  E  Y  T  F  E  C  I  G  N  V  G  T  M  R  A  A
 841 CTAGAAGCTTGCCATAAGGGATGGGGTGTGTCAGTGATCATTGGTGTAGCTGCTGCTGGA
     L  E  A  C  H  K  G  W  G  V  S  V  I  I  G  V  A  A  A  G
 901 GAAGAGATCAGCACTCGTCCATTCCAACTTGTTACAGGTCGCACCTGGAAGGGAACAGCT
     E  E  I  S  T  R  P  F  Q  L  V  T  G  R  T  W  K  G  T  A
 961 TTTGGAGGTTACAAAAGTAGAGAAAGTGTACCAAAGCTTGTAGATGAGTACTTGGAGAAG
     F  G  G  Y  K  S  R  E  S  V  P  K  L  V  D  E  Y  L  E  K
1021 AAGCTGCCTTTAGATGAATTTGTCACTCACAATGTGCCGCTGAAGGAGATCAATGAGGCA
     K  L  P  L  D  E  F  V  T  H  N  V  P  L  K  E  I  N  E  A
1081 TTCCATTTGATGCATGCTGGAAAATCTATCCGTGCAGTAGTTGACATGTAAtttctaagg
     F  H  L  M  H  A  G  K  S  I  R  A  V  V  A  M  *
1141 aaaaatatttatactatttttgtacacttgttaaacctttttttcttcagcatttactaca
1201 agtataattaagatacacaacatgcattttgtttacacaataaaaaaataacagaactga
1261 aaaataaaaaaaaaaaaaaa
```

Fig. 1. The nucleotide and deduced amino acid sequences of *B. mori* ADH III cDNA. The start codon ATG is boxed and the termination codon is shown by asterisk. The putative polyadenylation signal in the 3' UTR is underlined.

Results and Discussion

A cDNA library constructed using whole bodies of *B. mori* larvae was screened to generate ESTs. Of these ESTs, one clone had an insert of 1,385 bp, which contained the complete 3 and 5 ends. Sequences of the clone exhibited similarity to previously reported ADHs. The complete nucleotide and deduced amino acid sequences of *B. mori* ADH are presented in Fig. 1. An open reading frame of 1,128 bp was found, predicting a polypeptide of 376 amino acid residues with a calculated molecular mass of approximately 40 kDa.

The deduced amino acid sequence of *B. mori* ADH cDNA was aligned with other known ADHs. A multiple sequence alignment of the deduced protein sequence of *B. mori* ADH cDNA with other ADH sequences is shown in Fig. 2. Alignment of the *B. mori* ADH sequences with those of class III ADHs from several other species indicates the extent of the identity that exists. In addition, the *B. mori* ADH showed high protein sequence identity (87%–83%) to the calss III ADHs known from mammal, plant, fish, and insect, etc. (Table 2). Interestingly, the *B. mori* ADH showed higher homology to ADH III from fish [*Sparus aurata* (87% protein identity) and *Branchiostoma floridae* (85% protein identity)] rather than insect ADH III from *D. melanogaster* (83% protein identity). The deduced amino acid sequence of *B. mori* ADH is homologous with that of other class III ADH forms, reflecting its intermediary position from a branch between the mammal and lower vertebrate or invertebrate forms known. As

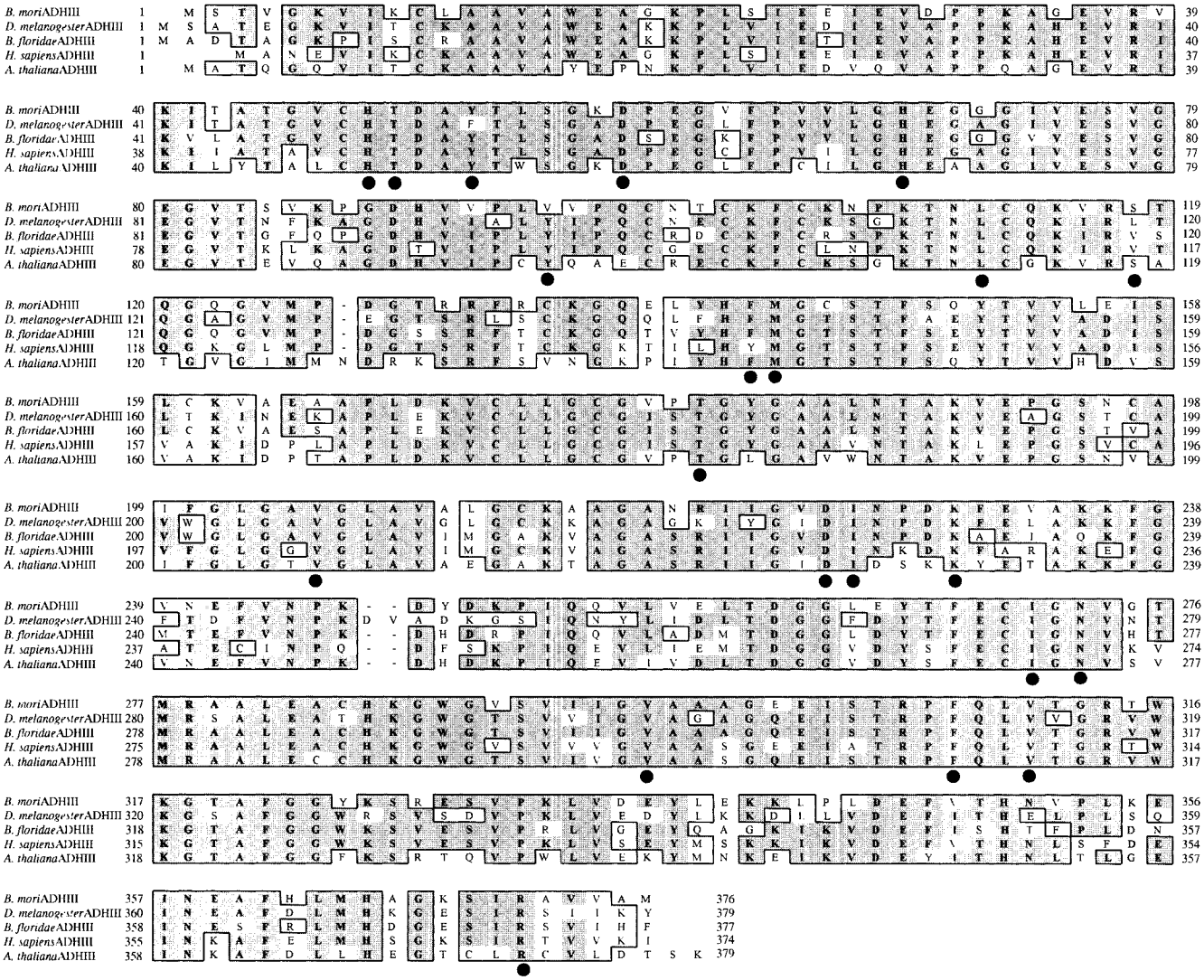

Fig. 2. Multiple sequence alignment of deduced protein sequences of the *B. mori* ADH III gene with other class III ADHs. The solid boxes are the residues that are identical to those of *B. mori*. Gaps have been introduced to obtain maximum alignment. Solid circles denote residues in substrate and coenzyme binding interactions.

Table 2. Pairwise identities and similarities of the deduced amino acid sequences among *B. mori* ADH III and other ADH genes

			Percent similarity																										
	Sequence	GenBank no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	*Bm*ADHIII	This study		87	85	85	84	85	83	83	83	82	83	81	28	24	24	24	24	24	23	244	23	23	16	16	17	15	17
2.	*Sa*ADHIII	U84719	76		87	89	90	90	85	85	84	87	79	29	25	25	26	25	25	24	25	25	25	23	18	18	19	19	20
3	*Bf*ADH3	AF154331	75	77		85	84	86	86	86	84	83	79	27	24	23	23	23	23	22	23	22	22	22	16	18	17	16	19
4	*Oc*ADH3	Y15406	74	82	74		96	95	81	81	81	94	77	29	24	24	24	24	24	23	24	23	23	22	16	17	18	17	18
5	*Hs*ADH5	M30471	73	81	73	94		97	80	80	82	96	78	28	23	23	24	23	23	233	23	23	23	22	16	17	18	17	18
6	*Mm*ADH2	M84147	72	81	73	91	92		82	82	82	94	78	28	24	23	24	23	23	23	24	23	23	22	17	17	18	16	18
7	*Dmel*FDH	AYO89518	71	74	75	71	69	71		100	81	79	78	26	24	23	23	23	23	22	23	22	23	22	15	17	17	15	16
8	*Dmel*ADH-F	U07641	71	74	75	71	69	71	100		81	79	78	26	24	23	23	23	23	22	23	22	23	22	15	17	17	15	16
9	*Ci*ADH3	AF344172	71	74	75	72	71	71	71	71		80	77	26	22	23	23	23	23	22	22	22	22	21	16	16	17	16	18
10	*Hs*FDH	XM_208352	71	77	70	91	95	89	68	68	69		76	26	23	23	23	23	23	23	23	23	22	21	17	17	17	16	17
11	*At*ADHIII	AY087250	67	67	65	65	67	67	64	64	62	65		27	22	22	22	22	22	21	22	22	22	19	16	16	16	16	16
12	*Db*ADH2	U65746	17	18	16	16	15	16	16	16	15	14	16		60	58	58	58	58	60	59	59	60	55	37	34	38	36	37
13	*Db*ADH1	U65746	11	13	11	11	10	11	12	12	9	10	10	53		93	94	94	94	94	94	96	95	89	49	48	50	50	55
14	*Dmon*ADH1	U26842	11	13	10	10	10	10	11	11	9	10	10	53	87		99	98	98	93	95	94	95	90	50	48	50	50	56
15	*Dmon*ADH2	U26845	11	13	10	10	10	10	11	11	9	10	10	53	88	99		99	99	93	94	95	95	90	50	49	50	50	56
16	*Dv*ADH1	U26846	11	13	10	10	10	10	11	11	9	10	10	53	88	96	97		100	93	95	95	95	90	49	48	50	50	55
17	*Dv*ADH2	U26846	11	13	10	10	10	10	11	11	9	10	10	53	88	96	97	100		93	95	95	95	90	49	48	50	50	55
18	*Dh*ADH2	X58694	11	13	10	10	10	10	11	11	9	10	10	55	90	88	88	88	88		93	96	93	89	48	47	49	49	55
19	*Dmoj*ADH1	X12536	11	13	11	11	10	11	11	11	9	10	10	54	91	90	90	90	90	90		95	96	90	51	48	51	51	57
20	*Dh*ADH1	X58694	11	13	10	10	10	10	11	11	9	10	10	55	91	90	91	90	90	95	91		96	90	48	47	50	48	55
21	*Dmoj*ADH2	X12536	11	13	10	10	10	10	11	11	10	9	10	55	90	90	91	91	91	90	93	92		89	50	48	51	50	58
22	*Dmel*ADH	AF175220	10	10	9	9	9	9	10	10	8	9	7	47	78	79	80	79	79	79	80	79	79		52	49	51	50	58
23	*Bo*ADH1	AJ277835	8	9	7	7	7	7	8	8	7	7	6	18	29	31	31	31	31	30	30	29	30	34		89	89	87	74
24	*Cc*ADH1	Z30194	7	8	7	7	7	6	8	8	6	7	6	19	31	32	33	33	33	32	31	31	31	33	81		86	85	73
25	*Cc*ADH2	Z30195	7	8	6	6	6	6	7	7	6	6	5	20	32	33	33	33	33	32	33	32	33	34	77	78		93	75
26	*Bo*ADH2	AJ277834	6	9	6	6	6	6	7	7	6	6	5	17	28	30	30	30	30	29	29	28	29	31	77	74	84		78
27	*Sp*ADH	D63669	6	6	6	5	5	6	6	6	5	5	5	18	33	33	33	33	33	32	34	31	34	36	53	52	57	56	

Percent identity

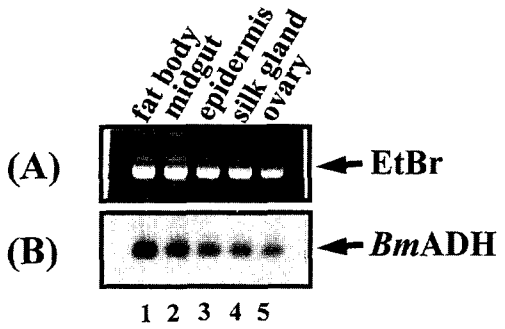

Fig. 3. Northern blot analysis of the *B. mori* ADH III messages. Total RNA was isolated from the fat body (lane 1), midgut (lane 2), epidermis (lane 3), silk gland (lane 4), ovary (lane 5). The RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (Al), transferred onto a nylon membrane, and hybridized with the appropriate radiolabeled probe (B). *B. mori* ADH III messages are indicated by arrow on the right side of the panel.

shown in Fig. 2, amino acid residues in substrate and coenzyme binding interactions were well conserved in the class III ADHs from mammalian, plant, fish and insect including *B. mori* (Hjelmqvist *et al.*, 1995). Therefore, these results suggested that *B. mori* ADH cloned in this study is class III ADH enzyme.Class III ADH, glutathione-dependent formaldehyde dehydrogenase, has been purified and characterized from various species such as mammalian, plant, fish and insect (Canestro *et al.*, 2000; Danielsson and Jornvall, 1992; Dasmahapatra *et al.*, 2001; Duester *et al.*, 1999; Hjelmqvist *et al.*, 1995; Funkenstein and Jakowlew, 1996; Shafqat *et al.*, 1996). Except for *Drosophila* ADH III, *B. mori* ADH III now determined is the first report in insect ADHs. Until now, furthermore, the genetic information of ADH has not been reported in any lepidopteran insects.

The ADH III mRNA expression in *B. mori* was determined by Northern blot analysis (Fig. 3). Total RNA was prepared from fat body, midgut, epidermis, silk gland, and ovary of *B. mori* larvae. Hybridization signals were detected from all tissues examined and the expression level of *B. mori* ADH III mRNA was the highest in the fat body.

Drosophila ADH III mRNA is present at all developmental stages of the fly (Danielsson *et al.*, 1994). The expression of ADH III in mammals is ubiquitous, with transcripts found in all tissues analyzed (Estonius *et al.*, 1996). ADH III mRNA in the marine teleost, *S. aurata*, was expressed in the eggs and embryos (Funkenstein and Jakowlew, 1996). This transcript distribution pattern would be consistent with its proposed housekeeping role in cytoprotection by metabolism of formaldehyde (Uotila and Koivusalo, 1989). In this study, *B. mori* ADH III was expressed in all tissues examined, such as fat body, midgut, epidermis, silk gland, and ovary of *B. mori* larvae. From these data, the present result suggests that *B. mori* ADH III distribution is ubiquitous and ADH III in *B. mori* larvae may play an important role in cellular metabolism. In conclusion, we firstly report the cDNA sequence and mRNA expression of ADH III in the silkworm, *B. mori*. The present result is the first case of elucidation of ADH gene in lepidopteran insect and will expand the understanding of insect ADH genes.

References

Atrian, S., L. Sánchez-Pulido, R. Gonzàlez-Duarte and A. Valencia (1998) Shaping of *Drosophila* alcohol dehydrogenase through evolution: relationship with enzyme functionality. *J. Mol. Evol.* **47**, 211-221.

Batterham, P., G. K. Chambers, W. T. Starmer and D. T. Sullivan (1984) Origin and expression of an alcohol dehydrogenase gene duplication in the genus *Drosophila*. *Genetics* **145**, 375-382.

Bayer, C. A., S. W. Curtiss, J. A. Weaver and D. T. Sullivan (1992) Delineation of cis-acting sequence required for expression of *Drosophila mojavensis Adh-1*. *Genetics* **131**, 143-153.

Begun, D. J. (1997) Origin and evolution of a new gene descended from alcohol dehydrogenase in *Drosophila*. *Genetics* **145**, 375-382.

Begun, D. J., A. J. Betancourt, C. H. Langley and W. Stephan (1999) Is the fast/slow allozyme variation at the *Adh* locus of *Drosophila melanogaster* an ancient balanced polymorphism? *Mol. Biol. Evol.* **16**, 1816-1819.

Benos, P., N. Tavermarakis, S. Brogna, G. Thireos and C. Savakis (2000) Acquisition of a potential marker for insect transformation: isolation of a novel alcohol dehydrogenase gene from *Bactrocera oleae* by functional complementation in yeast. *Mol. Gen. Genet.* **263**, 90-95.

Cañestro, C., R. Gonzalez-Duarte and R. Albalat (2002) Minisatellite instability at the *Adh* locus reveals somatic polymorphism in amphioxus. *Nucleic Acids Res.* **30**, 2871-2876.

Cañestro, C., L. Hjelmqvist, R. Albalat, J. Garcia-Fernandez, R. Gonzalez-Duarte and H. Jörnvall (2000) Amphioxus alcohol dehydrogenase is a class 3 form of single type and of structural conservation but with unique developmental expression. *Eur. J. Biochem.* **267**, 6511-6518.

Danielsson, O., S. Atrian, T. Luque, L. Hjelmqvist, R. Gonzàlez-Duarte and H. Jörnvall (1994) Fundamental molecular differences between alcohol dehydrogenase classes. *Proc. Natl. Acad. Sci. USA* **91**, 4980-4984.

Danielsson, O. and H. Jörnvall (1994) 'Enzymogenesis': classical liver alcohol dehydrogenase origin from the glutathione-dependent formaldehyde dehydrogenase line. *Proc. Natl. Acad. Sci. USA* **89**, 9247-9251.

Dasmahapatra, A. K., H. L. Doucet, C. Bhattacharyya and M. J. Carvan III (2001) Developmental expression of alcohol dehydrogenase (ADH3) in zebrafish (*Danio rerio*). *Biochem. Biophs. Res. Comm.* **286**, 1082-1086.

Duester, G., J. Farres, M. R. Felder, R. S. Holmes, J. O. Höög, X. Pares, B. V. Plapp, S. J. Yin and H. Jörnvall (1999) Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. *Biochem. Pharmacol.* **58**, 389-395.

Estonius, M., S. Svensson and J. O. Höög (1996) Alcohol dehydrogenase in human tissues: localization of transcripts coding for five classes of the enzyme. *FEBS Lett.* **397**, 338-342.

Funkenstein, B. and S. B. Jakowlew (1996) Molecular cloning of fish alcohol dehydrogenase cDNA. *Gene* **174**, 159-164.

Gasperi, G., L. Baruffi, A. Malacrida and A. S. Robinson (1992) A biochemical genetic study of alcohol dehydrogenase isozymes of the medfly *Ceratitis capitata. Biochem. Genet.* **30**, 289-304.

Gasperi, G., D. Kafetzopoulos, A. Christodoulidou, V. Bouriotis and C. Savakis (1994) Isolation and partial characterization of two alcohol dehydrogenase isozymes from the medfly *Ceratitis capitata. Insect Biochem. Molec. Biol.* **24**, 87-94.

Goulielmos, G. N., N. Cosmidis, M. Loukas, S. Tsakas and E. Zouros (2001) Characterization of two alcohol dehydrogenase (*Adh*) loci from the olive fruit fly, Bactrocera (*Dacus*) oleae and implications for *Adh* duplication in dipteran insects. *J. Mol. Evol.* **52**, 29-39.

Haas, B. J., N. Volfovsky, C. D. Town, M. Troukhan, N. Alexandrov, K. A. Feldmann, R. B. Flavell, O. White and S. L. Salzberg (2002) Full-length messenger RNA sequences greatly improve genome annotation. *Genome Biol.* **3**, 1-12.

Hjelmqvist, L., J. Shafqat, A. R. Siddiqi and H. Jörnvall (1995) Alcohol dehydrogenase of class III: consistent patterns of structural and functional conservation in relation to class I and other proteins. *FEBS Lett.* **373**, 212-216.

Horio, T., T. Kubo and S. Natori (1996) Purification and cDNA cloning of the alcohol dehydrogenase of the flesh fly *Sarcophaga penegrina.* A structural relationship between alcohol dehydrogenase and a 25-kDa protein. *Eur. J. Biochem.* **237**, 698-703.

Hur, M. W., W. H. Ho, C. J. Brown, D. Goldman and H. J. Edenberg (1992) Molecular cloning of mouse alcohol dehydrogenase-B2 cDNA: nucleotide sequences of the class III ADH genes evolve slowly even for silent substitutions. *DNA Seq.* **3**, 167-175.

Kim, I., K. S. Lee, B. R. Jin, Y. S. Lee and K. S. Ryu (2003a) cDNA sequence and mRNA expression of a putative alcohol dehydrogenase from the mole cricket, *Gryllotalpa orientalis. Int. J. Indust. Entomol.* **7**, 37-44.

Kim, S. R., K. S. Lee, I. Kim, S. W. Kang, S. K. Nho, H. D. Sohn and B. R. Jin (2003b) Molecular cloning of a cDNA encoding putative calreticulin from the silkworm, *Bombyx mori. Int. J. Indust. Entomol.* **6**, 93-97.

Koivusalo, M., M. Baumann and L. Uotila (1989) Evidence for the identity of glutathione-dependent formaldehyde dehydrogenase and class III alcohol dehydrogenase. *FEBS Lett.* **257**, 105-109.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Malacrida, A. R., G. Gasperi, A. Christodoulidou, C. Torti, E. Riva-Francos and R. Milani (1992) Evidence for a genetic duplication involving alcohol dehydrogenase genes in *Ceratitis capitata. Biochem. Genet.* **30**, 35-48.

Mazi, V. E., N. Cosmidis, Y. D. Clonis and M. Loukas (1998) Purification of alcohol dehydrogenase from four genotypes of the olive fruit fly *Bactrocera* (*Dacus*) *oleae. Biotech. Prog.* **14**, 294-299.

Menotti-Raymond, M., W. T. Starmer and D. T. Sullivan (1991) Characterizauon of the structure and evolution of the *Adh* region of *Drosophila hydei. Genetics* **127**, 355-366.

Nurminsky, D. I., E. N. Moriyama, E. R. Lozovskaya and D. L. Hartl (1996) Molecular phylogeny and genome evolution in the *Drosophila virilis* species group: duplication of the alcohol dehydrogenase gene. *Mol. Biol. Evol.* **13**, 132-149.

Shafqat, J., M. El-Ahmad, O. Danielsson, M. C. Martinez, B. Persson, X. Pares and H. Jörnvall (1996) Pea formaldehyde-active class III alcohol dehydrogenase: Common derivation of the plant and animal forms but not of the corresponding ethanol-active forms (classes I and P). *Proc. Natl. Acad. Sci. USA* **93**, 5595-5599.

Sharma, C. P., E. A. Fox, B. Holmquist, H. Jörnvall and B. L. Vallee (1989) cDNA sequence of human class III alcohol dehydrogenase. *Biochem. Biophys. Res. Comm.* **164**, 631-637.

Svensson, S., J. J. Hedberg and J. O. Höög (1998) Structural and functional divergence of class II alcohol dehydrogenase-cloning and characterization of rabbit liver isoforms of the enzyme. *Eur. J. Biochem.* **251**, 236-243.

Swofford, D. L. (2000) PAUP*. Phylogenetic analysis using parsimony (*and Other Methods), version 4, Sinauer Sunderland, MA.

Uotila L and M. Koivusalo (1989) Glutathione-dependent oxidoreductase: formaldehyde dehydrogenase; in *Coenzymes.* Vol. 3, Dolphin, D., R. Poulson and O. Avramovic (eds.), pp. 517-551, John Wiley and Sons, New York.