Int. J. Indust. Entomol. Vol. 4, No. 2, 2002, pp. 163~168

International Journal of
Industrial Entomology

Molecular Cloning of a cDNA Encoding a Ferritin Subunit from the Spider, *Araneus ventricosus*

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(Received 11 March 2002; Accepted 3 June 2002)

We report for the first time the cDNA sequence encoding a ferritin subunit from the spider, *Araneus ventricosus*. The complete cDNA sequence of *A. ventricosus* ferritin subunit comprised 516 bp with 172 amino acid residues. The *A. ventricosus* ferritin subunit cDNA contained a conserved iron responsive element sequence in the 5 untranslated region. An alignment of the deduced protein sequence of the *A. ventricosus* ferritin subunit gene to that of other heavy chain ferritin molecules showed that *A. ventricosus* ferritin subunit is most similar to the great pond snail, *Lymnaea stagnalis*, ferritin with 70.2% of protein sequence identity.

Key words : Spider, *Araneus ventricosus*, Ferritin, cDNA sequence, Iron storage protein

Introduction

Ferritin is the major intracellular repository of iron, and its synthesis is critical for iron metabolism. Ferritin is an iron storage protein ubiquitously distributed in animals, plants, as well as fungi and bacteria. The protein is generally composed of two subunits, termed heavy (H) and light (L) (Andrews *et al.*, 1992; Bomford and Munro, 1992; Lawson *et al.*, 1991).

Ferritin molecules have been isolated from numerous vertebrates and plants as well as several invertebrates (Theil, 1987). Most insect ferritins are larger (> 600 kDa) than vertebrate ones (~ 450 kDa), and insects have additional larger subunits (24 - 32 kDa) as well as small subunits homologous to the heavy and light protein chains of vertebrate ferritins (Huebers *et al.*, 1988; Nichol and Locke, 1989, 1999; Dunkov *et al.*, 1995; Winzerling *et al.*, 1995; Capurro *et al.*, 1996). Snail ferritin has two forms, S form (soma) and Y form (yolk) (von Darl *et al.*, 1994). Both snail ferritin sequences are more similar to H chain vertebrate ferritins than to L chain ferritins. Crayfish ferritin is approximately 50% similar to vertebrate H chain ferritin (Huang *et al.*, 1996). A ferritin molecule cloned from an echinoderm is involved with changes in iron concentrations carried out by the phagocytic coelomocytes (Beck *et al.*, 2002).

Although invertebrate ferritin genes have been isolated from several species, molecular information on spider ferritin has not yet been reported. The spider, *Araneus ventricosus,* is an abundant species in Korea (Kim *et al.*, 1997). The body size of *A. ventricosus* is relatively larger than that of other species. Thus, our aim is to elucidate the structure of ferritin gene in spider. In this study, we report for the first time the cDNA sequence for one subunit of ferritin from *A. ventricosus*.

Materials and Methods

cDNA library screening, nucleotide sequencing and data analysis

A cDNA library (Chung *et al.*, 2001) was constructed from the whole body of the spider, *Araneus ventricosus*. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). 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 homol-

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ogy using a BLAST algorithm program.

MacVector (ver. 6.5) was used to align the amino acid sequences of ferritin gene. Including the ten GenBank-registered amino acid sequences of ferritin subunit genes, phylogenetic analysis among ferritin subunit genes was performed using PAUP (Phylogenetic Analysis using Parsimony) version 3.0 (Swofford, 1990). The accession numbers of the sequences in the GenBank are as follows: spider *Araneus ventricosus* (AvFer; this study), great pond snail *Lymnaea stagnalis* (LsFerS; P42577; von Darl *et al.*, 1994), *Dermacentor variabilis* (DvFer; AF467696; Mulenga and Azad, 2002), soft tick *Ornithodoros moubata* (OmFer; AF068225; Kopacek *et al.*, 1998a), castor bean tick *Lxodes ricinus* (LrFer; AF068224; Kopacek *et al.*, 1998b), European house dust mite *Dermatophagoides pteronyssinus* (DpFerH; AY005807; Epton *et al.*, 2001), African clawed frog *Xenopus laevis* (XlFerH; P49948; Muller *et al.*, 1991), Atlantic salmon *Salmo salar* (SsFerH; P49946; Andersen *et al.*, 1995), human *Homo sapiens* (HsFerH; 2554682; Lawson *et al.*, 1991), chicken *Gallus gallus* (GgFerH; P08267; Stevens *et al.*, 1987), and house mouse *Mus musculus* (MmFerH; NM010239; Torti *et al.*, 1988).

The RNAfold program was used to predict the secondary structures of the RNA for the ferritin IRE (iron responsive element) (Beck *et al.*, 2002). Helical and non-helical regions of *A. ventricosus* ferritin were predicted by the Garnier-Robson method (Garnier *et al.*, 1978).

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-153                                GGAGAGAGAGAGAGAACTAGTCTCGAGTTTTTT
                 -104          IRE          -76
-120  TTTTTTGTTTTTTGGTGGTCGCGTCAA[redacted]TTGATCGAACACCTGTTCGTCTTTTTA
 -60  ACGATTTTTTAAGAGAGTTTTGAAGTTTGCTGCGTTTCTGTAAGAAAATCAGAACCAAAG

   1  ATGTCCAAGGGTAGCCAGATCCGCCAAAACTACCATGAGGAAAGTGAGGCTGGAGTCAAC
   1   M  S  K  G  S  Q  I  R  Q  N  Y  H  E  E  S  E  A  G  V  N
  61  AAGCAGATCAATATGGAGCTCTATGCTTCATATGTCTACGCTGCTATGGCCTTCCACTTC
  21   K  Q  I  N  M  E  L  Y  A  S  Y  V  Y  A  A  M  A  F  H  F
 121  GACCGGGATGACGTAGCTTTGCCTAACATCTCTCAGTTCTTCAAGGAGAACAGCGATGAG
  41   D  R  D  D  V  A  L  P  N  I  S  Q  F  F  K  E  N  S  D  E
 181  GAGAAGGAACATGCCAACAAGTTGATGAAGTTCCAGAACCAAAGGGGTGGAACTATTGTG
  61   E  K  E  H  A  N  K  L  M  K  F  Q  N  Q  R  G  G  T  I  V
 241  CTGAAAGACATCAAGGCTCCTCCCAAGGCAAAGTGGGGCTCTCCCCTCGAGGCCTTCCAA
  81   L  K  D  I  K  A  P  P  K  A  K  W  G  S  P  L  E  A  F  Q
 301  GATGCTCTTGAACTGGAAAAGACTGTCAATCAAGCCTTGCTGGATTTGCATAAGGTCGCC
 101   D  A  L  E  L  E  K  T  V  N  Q  A  L  L  D  L  H  K  V  A
 361  TCCAGTCACGATGACGCGCAGATGTGCGACTTCTTGGAAAGTGAATACCTGACTGAGCAA
 121   S  S  H  D  D  A  Q  M  C  D  F  L  E  S  E  Y  L  T  E  Q
 421  GTGGAGGCTATCAAGAAGCTCGGAGACTACGTCACCAACTTGAAGCGTGTTGGTTCTGGC
 141   V  E  A  I  K  K  L  G  D  Y  V  T  N  L  K  R  V  G  S  G
 481  CTGGGCGAGTTCATCTTCGACAAGGAATTCAAATCTTAAAACAGCTTTTTATTCCTTTAC
 161   L  G  E  F  I  F  D  K  E  F  K  S  *
 541  AATTCCTCTTGTTAAACAACGCAAAGGAATTCAAAACTGTAATTCACCTTAGAATTAAAA
 601  TTTAAACCTACCAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of *A. ventricosus* ferritin subunit gene. The start codon of ATG is boxed and the termination codon is asterisked. The putative iron response element (IRE) in the 5' untranslated region (UTR) is indicated by the double lines. Contained within the IRE is the conserved six-membered loop structure (5'-CAGUGU-3', underlined) of the IRE. The putative polyadenylation signal in the 3' UTR is underlined.

Results and Discussion

A cDNA library was constructed from *A. ventricosus* whole body RNA (Chung *et al.*, 2001). The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the *A. germari* ESTs. Of these ESTs, one exhibited similarity to the reported ferritin subunits. This clone was selected for further characterization.

The nucleotide and deduced amino acid sequences of the cDNA encoding the *A. ventricosus* ferritin subunit are presented in Fig. 1. The complete subunit cDNA sequence comprised of 516 bp with 172 amino acid residues with a calculated molecular mass of approximately 20 kDa. The *A. ventricosus* ferritin subunit cDNA contains a 153 base

A)

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Consensus        TCTTGCTTCAACAGTGTTTGAACGGAACC

AvFer      -104  GG-C--G--------------T--A-CA-  -76
LsFerS           -------G-GT-----AAC-T--A---AT
HsFerH           --C-----------------G--------
GgFerH           --C-------------C---G-------G
```

B)

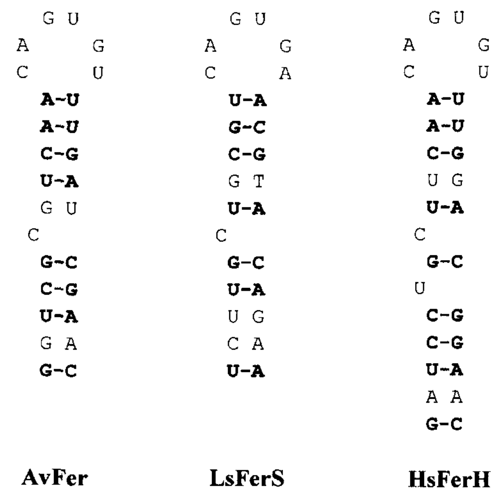

Fig. 2. Alignment of IRE from sequences of several ferritin 5' UTRs. (A) Alignment of the cDNA sequence of the *A. ventricosus* ferritin subunit IRE with other ferritin IREs. The nucleotide positions are relative to the first ATG codon. A hyphen in the sequence indicates identity with the consensus. AvFer, *A. ventricosus* ferritin subunit; LsFerS, *L. stagnalis* soma form ferritin; HsFerH, *H. sapiens* heavy chain ferritin; GgFerH, *G. gallus* heavy chain ferritin. (B) Predicted secondary structure in the 5' UTR sequence of the AvFer IRE with comparison to the LsFerS IRE and the HsFerH IRE.

5' untranslated region (UTR) followed by an open reading frame of 172 amino acids and a 110 base 3' UTR containing a putative polyadenylation site. Shown in Fig. 1 and in more detail in Fig. 2 are the 5' UTR of the *A. ventricosus* ferritin subunit cDNA. Examination of the 5 UTR of the *A. ventricosus* ferritin subunit cDNA revealed a sequence similar to the iron responsive element (IRE) sequence found in other ferritins. It contained a highly conserved IRE sequence in positions 104 to 76 (Fig. 1 and Fig. 2A). The IREs have been found in the majority of ferritin mRNAs (Beck *et al.*, 2002; Charlesworth *et al.*, 1997; von Darl *et al.*, 1994; Dunkov *et al.*, 1995; Drysdale, 1988; Huang *et al.*, 1996, 1999; Pham *et al.*, 1996; Theil, 1987). The region predicts a secondary stem-loop structure, which contains a typical invariant six-nucleotide loop (CAGUGN), very similar to vertebrate ferritin IRE (Andrews *et al.*, 1992; Huang *et al.*, 1996, 1999; Theil, 1987). As shown in Fig. 2B, the loop is followed by a 5 bp stem and a bulged 5 cytosine. This is very similar to the vertebrate and invertebrate ferritins (Andrews *et al.*, 1992; Beck *et al.*, 2002; Charlesworth *et al.*, 1997; Gray *et al.*, 1996; Theil, 1987).

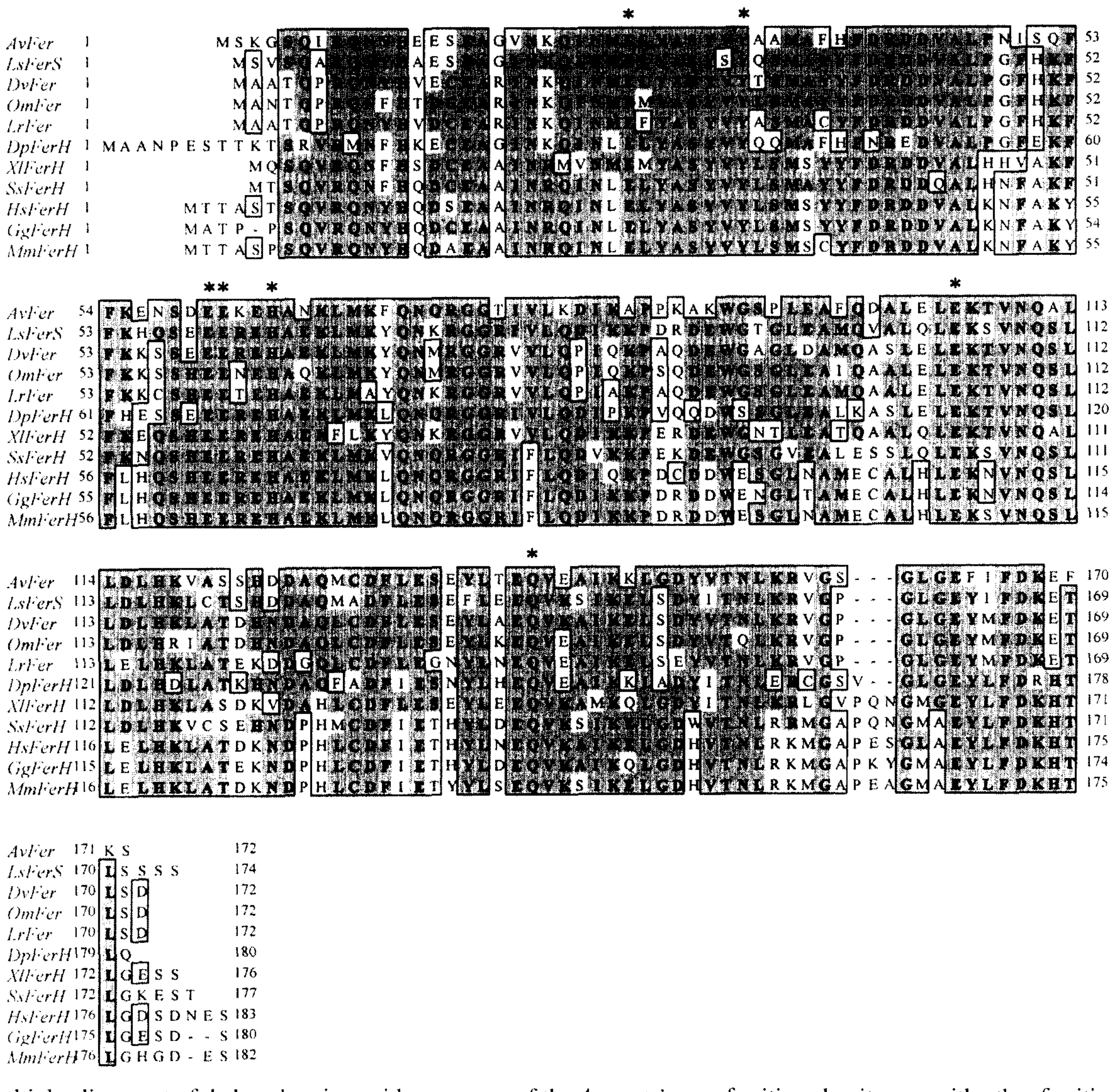

Fig. 3. Multiple alignment of deduced amino acid sequences of the *A. ventricosus* ferritin subunit gene with other ferritins. In solid box are the residues that are identical to those in the *A. ventricosus* ferritin subunit. The seven residues associated with the metal binding site are indicated by the asterisks. AvFer, *A. ventricosus* ferritin; LsFerS, *L. stagnalis* soma form ferritin; DvFer, *D. variabilis* ferritin; OmFer, *O. moubata* ferritin; LrFer, *L. ricinus* ferritin; DpFerH, *D. pteronyssinus* H chain ferritin; XlFerH, *X. laevis* H chain ferritin; SsFerH, *S. salar* H chain ferritin; HsFerH, *H. sapiens* H chain ferritin; GgFerH, *G. gallus* H chain ferritin; MmFerH, *M. musculus* H chain ferritin.

Table 1. Pairwise comparisons among amino acid sequences of the *A.ventricosus* ferritin subunit gene and the known ferritin genes

Species	GenBank No.	1	2	3	4	5	6	7	8	9	10	11
1. AvFer		-	0.298	0.303	0.309	0.319	0.367	0.383	0.404	0.447	0.447	0.452
2. LsFerS	P42577	56	-	0.197	0.245	0.245	0.362	0.287	0.314	0.351	0.351	0.346
3. DvFer	AF467696	57	37	-	0.101	0.122	0.324	0.303	0.346	0.351	0.362	0.372
4. OmFer	AF068225	58	46	19	-	0.138	0.346	0.298	0.351	0.372	0.388	0.394
5. LrFer	AF068224	60	46	23	26	-	0.372	0.324	0.372	0.362	0.367	0.372
6. DpFerH	AY005807	69	68	61	65	70	-	0.426	0.404	0.410	0.410	0.420
7. XlFerH	P49948	72	54	57	56	61	80	-	0.271	0.340	0.298	0.335
8. SsFerH	P49946	76	59	65	66	70	76	51	-	0.250	0.234	0.245
9. HsFerH	2554682	84	66	66	70	68	77	64	47	-	0.096	0.074
10. GgFerH	P08267	84	66	68	73	69	77	56	44	18	-	0.096
11. MmFerH	NM_010239	85	65	70	74	70	79	63	46	14	18	-

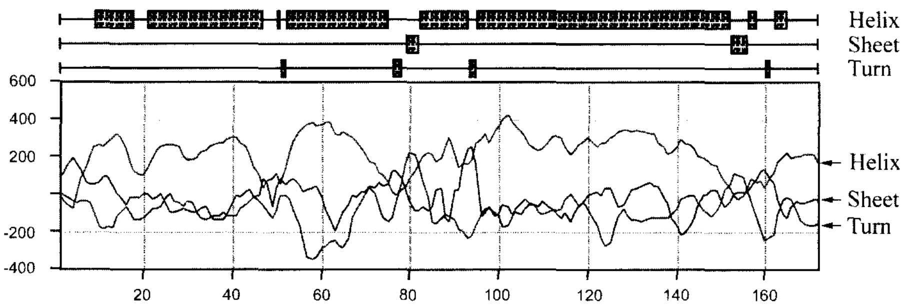

Fig. 4. Predicted helical regions of *A. ventricosus* ferritin subunit. Helical and non-helical regions of *A. ventricosus* ferritin subunit were predicted by the Garnier-Robson method (Garnier *et al.*, 1978).

Fig. 3 shows a multiple sequence alignment of the deduced protein sequence of *A. ventricosus* ferritin subunit gene with other vertebrate and invertebrate ferritin sequences. The deduced protein *A. ventricosus* ferritin subunit sequence showed significant sequence homologies with those of other heavy chain (H) ferritin molecules. The seven putative ferroxidase center residues associated with the metal-binding site (Andrews *et al.*, 1992; Beck *et al.*, 2002; Huang *et al.*, 1996; Theil, 1987) are conserved in the subunits among the species aligned.

The degree of similarity of several representive frritin molecules is shown in Table 1. The *A. ventricosus* ferritin subunit is most similar to the great pond snail (*L. stagnalis*) S form ferritin (70.2% protein sequence identity). Furthermore, the *A. ventricosus* ferritin subunit showed significantly protein sequence identity to the *D. variabilis* ferritin (69.7%), *O. moubata* ferritin (69.1%), *L. ricinus* ferritin (68.1%), *D. pteronyssinus* H ferritin (63.3%) and *X. laevis* H ferritin (61.7%).

The five α helices of *A. ventricosus* ferritin subunit are predicted as shown in Fig. 4.

This structure is very similar to the majority of ferritins. The ferritin molecule consists of 24 subunits with five helices which fold as four-helix bundles (Ha *et al.*, 1999; Hempstead *et al.*, 1997).

In conclusion, we report the cDNA sequence of ferritin H subunit in the spider, *A. ventricosus*. In this study, molecular characterization of the *A. ventricosus* ferritin subunit will expand our understanding on the invertebrate ferritins.

Acknowledgements

This paper was supported by the Dong-A University Research Fund in 2001.

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