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Characterization and expression of chicken selenoprotein W

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Abstract As an essential trace element, selenium (Se) deficiency results in White Muscle Disease in livestock and Keshan disease in humans. The main objectives of this study were to clone and characterize the chicken selenoprotein W (SeW) gene and investigate SeW mRNA expression in chicken tissues. The deduced amino acid (AA) sequence of chicken SeW contains 85 AAs with UAG as the stop codon. Like all SeW genes identified in different species, chicken SeW contains one well-conserved selenocysteine (Sec) at the 13th position encoded by the UGA codon. The proposed glutathione (GSH)-binding site at the Cys_{37} of SeW is not conserved in the chicken, but Cys_9 and Sec_{13}, with possible GSH binding, are conserved in SeWs identified from all species. There are 23–59% and 50–61% homology in cDNA and deduced AA sequences of SeW, respectively, between the chicken and other species. The predicted secondary structure of chicken SeW mRNA indicates that the selenocysteine insertion sequence element is type II with invariant adenosines within the apical bulge. The SeW mRNA expression is high in skeletal muscle followed by brain, but extremely low in other tissues from chickens fed a commercial maize-based diet. The SeW gene is ubiquitously expressed in heart, skeletal muscle, brain, testis, spleen, kidney, lung, liver, stomach and pancreas in chickens fed a commercial diet supplemented with sodium selenite. These results indicate that dietary selenium supplementation regulates SeW gene expression in the chicken and skeletal muscle is the most responsive tissue when dietary Se content is low.

Keywords Selenoprotein W · mRNA expression · Selenium · cDNA sequence · Chicken

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Introduction

As an essential trace element, selenium (Se) is important for animal and human health. Se deficiency causes cardiac and muscular degeneration, such as White Muscle Disease (WMD) in livestock and Keshan disease in humans (Schubert et al. 1961; Chen et al. 1980) and selenium supplementation reverses muscle weakness in Se-deficient humans and animals (Brown et al. 1986; Chariot and Bignani

2003). Se protects against gizzard myopathy and skeletal muscle degeneration, and increases reproductive performance in poultry (Walter and Jensen 1963; Cantor et al. 1978; Ebeid 2009). In addition, the recent report indicated that Se deficiency in the chicken impaired thymus function (Peng et al. 2010), thus Se supplementation is necessary for poultry growth performance.

Se exerts its biological function as selenoproteins in living organisms. Selenoproteins are known to be involved in various biological functions, such as antioxidation, immune responses, peroxide detoxification, selenium transport, thyroid function and cell cycle progression (Chung et al. 2009; Hawkes et al. 2009; Wang et al. 2010). Selenoproteins are Se-containing proteins in which Se is incorporated translationally as selenocysteine (Sec), synthesized from serine, by the UGA codon and Sec is found to be the active site in the selenoproteins of known function (Sunde and Evenson 1987; Whanger 2009). Selenocysteine insertion sequence (SECIS) element located in the 3-untranslated region of eukaryotic selenoprotein mRNAs is necessary for interpretation of the UGA codon as a Sec codon. Although the nucleotide sequences of SECIS elements are not conserved, the secondary structure of eukaryotic SECIS elements are fully conserved across different selenoprotein genes. A quartet (5′-UGAN and NGAN-3′) containing non-Watson–Crick base-pairing, a helix with GU/UG base pairs, and the unpaired invariant adenosines within the apical loop are essential parts of a SECIS element (Korotkov et al. 2002; Latreche et al. 2009). The SECIS element forms a conserved stem-loop secondary structure, and three types of SECIS element have been found in eukaryotes. The type I SECIS element, found in cellular glutathione peroxidase (cGPX), selenoprotein P (SeP) and type I iodothyronine deiodinase, contains 2–3 invariant adenosines within the apical loop consisting of approximately 9–16 nucleotides. In general, there are 10–12 nucleotides between the invariant adenosines and the quartet (Low and Berry 1996; Walczak et al. 1996; Xu et al. 2001). The type II SECIS element, found in selenoprotein W (SeW), phospholipid hydroperoxide GPX (phGPX), plasma GPX and type III iodothyronine deiodinase, contains a bulge loop and the upstream top loop within apical loop. The invariant adenosines are within the 5′-end of the bulge loop and there are generally 3–4 base pairs spacing the bulge loop from the upstream top loop (Low and Berry 1996; Walczak et al. 1996; Xu et al. 2001). A new form of SECIS element found in selenoprotein M (SeM) is similar to the type II SECIS with the cytosines (CC) replacing the invariant adenosines in the apical bulge loop (Korotkov et al. 2002).

There are at least 25 identified mammalian selenoproteins and the chicken selenoproteome consists of at least 23 Selenoproteins (Lobanov et al. 2008). The glutathione peroxidase (GPX) family was the first selenoprotein family identified with known function (Rotruck et al. 1973). Other selenoproteins include thioredoxin reductases, iodothyronine deiodinases, SeW, SeP, SeM, Selenoprotein N, selenoprotein H, selenoprotein T and others (Lu and Holmgren 2009). Although the function for many identified selenoproteins is still not confirmed, such as SeW, most selenoproteins are found to contain specific structure motifs to suggest their biological function to be involved in antioxidative or redox-related reactions (Ferguson et al. 2006; Aachmann et al. 2007; Dikiy et al. 2007). The function of SeW has been suggested to be involved in antioxidation and cell cycle progression (Jeong et al. 2002; Chung et al. 2009; Hawkes et al. 2009; Wang et al. 2010).

SeW has been found to be extremely low in skeletal muscles of WMD lambs (Pedersen et al. 1972), and is the 10-kDa selenoprotein containing one selenocysteine (Sec) encoded by the UGA codon (Vendeland et al. 1995). SeW is present in skeletal muscles at higher levels than in other tissues from rat, sheep, monkey and human (Yeh et al. 1995, 1997a; Gu et al. 2000; Bellingham et al. 2003). The cDNA sequences for SeW from mouse, rat, human, monkey, sheep, pig and zebrafish were identified and the Sec of SeWs found in different species is all well conserved at the 13th position of amino acid sequence (Gu et al. 1997). Since there is no available information for poultry SeW, the objectives of this study were to clone and characterize chicken SeW cDNA, and investigate the SeW mRNA distribution in various chicken tissues.

Materials and methods

Materials

Sodium selenite, agarose, formaldehyde, tris-acetate-ethylenediaminetetraacetic acid (TAE) buffer, and

ethidium bromide (EtBr) solution were purchased from Sigma Chemical Co. (St. Louis, MO USA). Invitrogen (Carlsbad, CA USA) was the source for TRIzol® Reagent, and Takara Bio USA (Madison, WI USA) was the source for Reverse Transcription-Polymerase Chain Reaction (RT-PCR) kit. The commercial maize-based diet was purchased from Fwusow Industry Co. (Taichung, Taiwan ROC) and the primers were synthesized by MDBio, Inc. (Taipei, Taiwan ROC).

Animal treatment

Five-month-old male Red-Feather Taiwan Country chickens were purchased locally (Taichung, Taiwan ROC) and fed a commercial maize-based diet containing 0.116 mg feed-derived Se/kg diet with or without addition of 4 mg Se/kg diet as sodium selenite for 10 days. Feed and tap water were supplied ad libitum. At the end of experiment, the animals were sacrificed and ten tissues (heart, skeletal muscle, brain, testis, spleen, kidney, lung, liver, stomach and intestine) were collected and immediately frozen at −80°C for later analysis. This animal research and all procedures used with the animals were reviewed and approved by the Animal Care and Use Committee at Asia University, Taichung, Taiwan ROC.

Total RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA was extracted from various chicken tissues by using TRIzol® Reagent following the manufacturer's protocol. The RNA concentration was determined by a DU Series 60 Spectrophotometer (Beckman Instruments, Fullerton, CA USA) at 260 nm, and the RNA quality was further confirmed by formaldehyde-agarose gel electrophoresis prior to RT-PCR analysis. The specific primers for chicken SeW were designed based on the sequence of expressed-sequence-tag (EST) clone, ChEST161o15 (5′) (GenBank Accession: BU411236; Boardman et al. 2002), by using GCG Seq Web 2.02 software (Accelrys Software Inc., San Diego, CA USA). The ABI 9700 thermocycler (Applied Biosystems, Foster City, CA USA) was used to perform RT-PCR analysis. The forward and reverse chicken SeW primers were 5′-AGATGCGCGGCCAGGGCA-3′ and 5′-GGCGTCAAACGAAACATTGA-3′, respectively. The PCR condition was initiated at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 54.8°C for 1.5 min and 72°C for 1 min, and extended by 72°C for 7 min at the end. Beta-actin was used as the internal control and the specific primers were designed based on the published exon sequence of the chicken β-actin gene (Kost et al. 1983). The forward and reverse primers of chicken β-actin were 5′-GAAGCCCAGAGCAAAAGAG-3′ and 5′-ACCAGAGTCCATCACAATACC-3′, respectively. The PCR condition was initiated by 94°C for 5 min, followed by 24 cycles of 94°C for 30 s, 54.8°C for 1.5 min and 72°C for 1 min, and extended by 72°C for 7 min at the end. The PCR products were electrophoresed on TAE agarose gels, stained with EtBr solution, then imaged under ultraviolet-light source. The PCR products for chicken SeW and β-actin were estimated to be approximately 500 and 300 base pairs, respectively.

Comparison and structure analysis of chicken SeW sequences

The amplified PCR product from chicken skeletal muscle was sequenced and the resulting chicken SeW sequence was confirmed by alignment with the published chicken genome sequences. The cDNA and the deduced amino acid sequences of chicken SeW were compared with seven different species. Except for chicken SeW sequence obtained in this study, the GenBank Accession numbers for mouse, rat, human, monkey, sheep, pig and zebrafish are U67890, U25264, U67171, NM_001042371, U67853, NM_213977 and AY216582, respectively. The secondary structure of chicken SeW mRNA was predicted by mfold (version 3.2) web server (Mathews et al. 1999; Zuker 2003) and RNA Draw program (Matzura and Wennborg 1996).

Results

Sequence and structure of chicken SeW

The cDNA sequence of chicken SeW from skeletal muscle is shown in Fig. 1, and the start codon (ATG), selenocysteine codon (TGA), stop codon (TAG), SECIS element stem-loop region and polyadenylation signal are indicated (Fig. 1). The primary structures

```
1   ATGCCGCTCC GCGTCACCGT GCTCTACTGC GGAGCCTGAG GCTACAAGCC
51  CAAGTACGAA CGGCTGCGGG CGGAGCTGGA GAAGCGCTTC CCCGGAGCGC
101 TGGAGATGCG CGGCCAGGGC ACGCAGGAGG TGACGGGATG GTTCGAGGTG
151 ACGGTGGGCA GCCGCCTGGT GCACTCCAAG AAGAACGGCG ACGGCTTCGT
201 GGACACCAAC GCCAAACTGC AGCGCATCGT GGCCGCCATC CAAGCTGCCC
251 TCCCGTAGGC CCCACATCGC CCCACATCCA ACGGGGCCAT GGGGGCCCAC
301 GCTGACCCCC CAGCACCGCC CGGCTCTGCC GGCACAGCGC TCCATGACGG
351 GGGTGGCCAA AAAGCCCACG GGCACAGGGC TGCTTCCAGA ACCCGCGCAC
401 GGCACCGGAG CCAGAGCTCG CGTTCCAACC TGGTGTGGGT CTGCTTTACG
451 CCATCGATGC TTCACTCTGT GTCATCCAGC TTTGGCGCAC GCCGTCCACG
501 TTTGACTTTA TGACACAGAG GTTTTGTTTG ACGCCATCGA CGCTTCGTTT
551 GACGCCATCG ACGTTTCATT TGACGCCATT GATGTTTCGT TTGACGCCAT
601 CGACGTTCTG CTTCATGCCC ACATCGCTCA CCGAATGCTG CTCCTCTCTG
651 GGTTGAGCTG TTCTGCCATT ATAACACCAC TCGGTGACGA TTCTCAATAA
701 A
```

Fig. 1 cDNA sequence of chicken SeW. Start codon (ATG), selenocysteine codon (TGA) and stop codon (TAG) are underscored. SECIS element stem-loop region is specified by the *rectangle box* and the *shed area* indicates polyadenylation signal (AATAAA)

of SeW genes in rat (Whanger et al. 1997) and chicken are listed with nucleotide position indicated (Fig. 2). The coding sequence and the deduced amino acid sequence of selenoprotein W from eight different species including mouse, rat, human, monkey, sheep, pig, zebrafish and chicken, are shown in Figs. 3 and 4, respectively. The SeW gene in all the species identified so far contains one selenocysteine (Sec) residue encoded by the UGA codon (Fig. 3), and the Sec residue for all species is located at the 13th position of the amino acid sequence (Fig. 4). The chicken SeW protein contains 85 amino acids and uses UAG as the stop codon to terminate translation (Fig. 4). The SeW sequences from eight different species were compared. There is 23–59% and 50–61% homology in SeW cDNA sequence and deduced amino acid sequences respectively, between chicken and other species including mouse, rat, human, monkey, sheep, pig and zebrafish (Table 1).

The secondary structure of chicken SeW mRNA was predicted by mfold version 3.2 web server (Fig. 5a) and RNA Draw program (Fig. 5b), and the corresponding primary structure of chicken SeW mRNA is listed with key positions indicated (Fig. 5c). The putative secondary structure of SECIS element in chicken SeW by RNA Draw program indicated that it is a type II SECIS element with

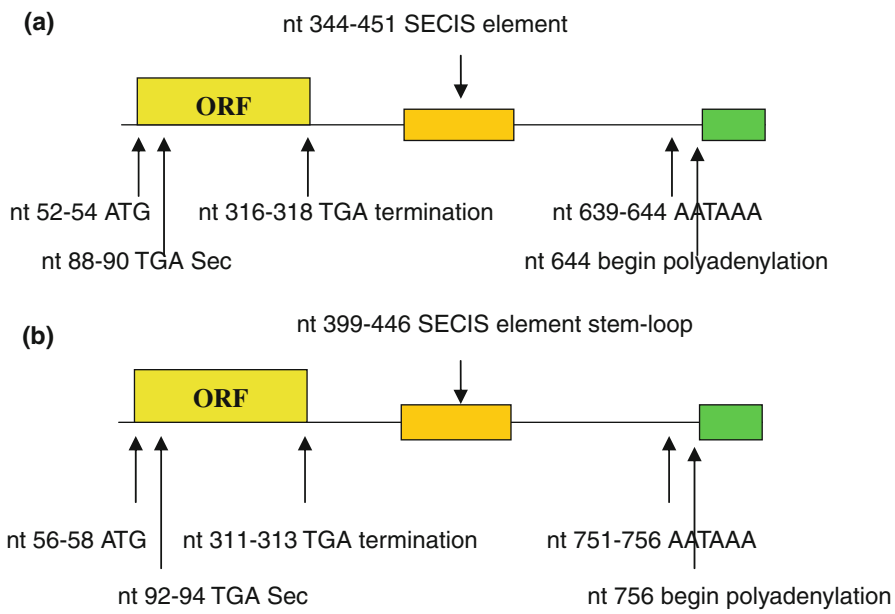

Fig. 2 Comparison of SeW gene primary structure between rat (**a**) (Whanger et al. 1997) and chicken (**b**)

Mouse	ATG	GCG	CTC	GCC	GTT	CGA	GTC	GTG	TAT	TGT	GGA	GCT	TGA	GGC	TAT	AAG	CCC	AAG	TAC	CTC
Rat	ATG	- - -	- - A	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	TGA	- - -	- - -	- - -	- - -	- - -	- - T	- - -
Human	ATG	- - T	- - -	- - -	- - C	- - -	- - -	- - T	- - -	- - -	- - C	- - -	TGA	- - -	- - C	- - -	T - -	- - -	- - T	- - T
Monkey	ATG	- - T	- - -	- - -	- - G	- - -	- - -	- - T	- - -	- - -	- - C	- - -	TGA	- - -	- - C	- - -	T - -	- - -	- - T	- - T
Sheep	ATG	- - -	G - -	- T -	- - C	- - -	- - A	- - T	- - -	- - -	- - C	- - -	TGA	- - -	- - C	- - -	- - -	- - -	- - T	- - T
Pig	ATG	- GT	G - -	- - -	- - -	- - -	- - -	- - C	- - -	- - -	- - C	- - -	TGA	- - -	- - C	- - -	T - -	- - -	- - T	- - T
Zebrafish	ATG	A - C	G - -	AAA	- - -	- AT	- - -	- - T	- - C	- - C	- - C	- GA	TGA	- - G	- - C	CG -	- - -	- - -	- T -	A - -
Chicken	ATG	C - -	- - -	CG -	- - C	ACC	- - G	C - C	- - C	- - C	- - -	- - C	TGA	- - -	- - C	- - -	- - -	- - -	- - -	GAA
Mouse	CAG	CTC	AAG	GAG	AAG	CTA	GAA	CAT	GAG	TTC	CCC	GGA	TGC	CTG	GAC	ATT	TGT	GGC	GAG	GGG
Rat	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - C	- - -	- - -	- - -	- - -
Human	- - -	- - -	- - -	A - -	- - -	T - -	- - -	G - -	- - -	- - -	- - -	- - C	C - -	- - -	- - -	- - C	- - C	- - -	- - -	- - A
Monkey	- - -	- - -	- - -	A - -	- - -	T - -	- - -	G - -	- - -	- - -	- - -	- - C	C - -	- - -	- - -	- - C	- - C	- - -	- - -	- - A
Sheep	- - -	- - -	- - -	A - -	- - -	T - -	- - -	G - -	- - -	- - -	- - T	A - C	C - T	T - -	- - -	- - C	- - C	- - -	- - -	- - -
Pig	- - A	- - -	- - -	A - -	- - -	T - -	- - -	G - -	- - -	- - -	- - T	- - -	C - -	- - -	- - -	- - C	- - -	- - -	- - -	- - -
Zebrafish	A - A	- - -	- - -	AC -	TT -	- - T	- - G	G - -	- - A	- - -	- - -	AAC	GAA	- - T	- - G	- - -	AC-	- - T	C - -	- - C
Chicken	- G -	- - G	CG -	- C -	G - -	- - G	- - G	A - G	CGC	- - -	- - -	- - -	GCG	- - -	- - G	- - G	C - C	- - -	C - -	- - C
Mouse	ACT	CCC	CAG	GTC	ACC	GGG	TTC	TTT	GAA	GTG	ACA	GTA	GCC	GGG	AAG	TTG	GTC	CAC	TCC	AAG
Rat	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - G	- - -	- - -	- - -	- - -	- - -	- - T	- - -	- - -	- - -
Human	- - -	- - -	- - -	- C -	- - -	- - -	- - -	- - -	- - -	- - -	- TG	- - -	- - -	- - -	- - -	- - -	A - T	- - -	- - -	- - -
Monkey	- - -	- - -	- - -	- C -	- - -	- - -	- - -	- - -	- - -	- - A	- TG	- - -	- - -	- - -	- - -	- - -	A - T	- - -	- T -	- - -
Sheep	- - -	- - -	- - -	- - -	- - -	- - C	- - -	- - -	- - -	- - -	TTC	- - -	- - G	- - A	- - -	C - -	- - T	- - -	- - -	- - -
Pig	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- C	- - -	- - A	TTG	- - -	- - A	- - -	- - -	- - -	- - T	- - -	- - -	- - -
Zebrafish	- - A	- - -	TC -	AC -	- - A	- - C	- GG	- - G	- - G	- - -	GA -	- - -	AA -	- - C	- - -	- - -	- - G	- - -	- - T	- - -
Chicken	- - G	- AG	G - -	- - G	- - G	- - A	- GG	- - C	- - G	- - -	- - G	- - G	- G -	A - C	CGC	C - -	- - G	- - -	- - -	- - -
Mouse	AAG	AGA	GGT	GAT	GGC	TAT	GTG	GAT	ACA	GAG	AGC	AAG	TTC	CGG	AAA	CTG	GTG	ACC	GCC	ATC
Rat	- - -	- - -	- - -	- - -	- - -	- - C	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - T	- - -	- - -
Human	- - -	- A -	- - C	- - -	- - -	- - C	- - -	- - C	- - -	- - A	- - -	- - -	- - T	- T -	- - G	T - -	- - -	G - -	- - -	- - -
Monkey	- - -	- A -	- - C	- - -	- - -	- - C	- - -	- - C	- - -	- - A	- - -	- - -	- - T	- T -	- - G	- - -	- - -	G - -	- - -	- - -
Sheep	- - -	G - -	- - C	- - -	- - -	- - C	- - -	- - C	- - G	- - -	- - -	- - -	- - T	- T -	- - G	- - -	- - -	G - -	- - -	- - -
Pig	- - -	G - -	- - -	- - -	- - -	- - C	- - -	- - -	- - G	- - -	- - -	- - -	- - T	- T -	- - G	T - -	- - -	G - T	- - -	- - -
Zebrafish	- - -	- AC	- - A	- - -	- - A	- TC	- - C	- - C	T - C	- - T	TCA	- - A	A - G	- A -	- - -	A - C	- - -	- - T	- - -	- - -
Chicken	- - -	- AC	- - C	- - C	- - -	- TC	- - -	- - C	- - C	A - C	GC -	- - A	C - G	- A -	CGC	A - C	- - -	G - -	- - -	- - -
Mouse	AAA	GCT	GCC	TTG	GCT	CAG	TGC	CAG	TGA											
Rat	- - -	- - C	- - -	- - -	- - -	- - -	- - -	- - -	TGA											
Human	- - -	- - C	- - -	- - -	- - -	- - -	G - -	TAA												
Monkey	- - -	- - C	- - -	- - -	- - C	- - -	G - -	TAA												
Sheep	- - -	- - C	- - T	- - -	- - -	- - -	GC-	TGA												
Pig	- - -	- - -	- - T	- - -	- - -	- - -	G - -	TAA												
Zebrafish	G-G	CAG	- - -	A - -	-GG	A - A	TGA													
Chicken	C - -	- - -	- - -	C-C	C-G	TAG														

Fig. 3 The SeW coding sequences of mouse, rat, human, monkey, sheep, pig, zebrafish and chicken. The mouse coding sequence is shown and the chicken coding sequence is sheded. *Dash line* indicates the same nucleotide at the same position as the mouse sequence, and only different nucleotides at the same position are shown

Mouse	M	A	L	A	V	R	V	V	Y	C	G	A	U	G	Y	K	P	K	Y	L
Rat	M	A	L	A	V	R	V	V	Y	C	G	A	U	G	Y	K	P	K	Y	L
Human	M	A	L	A	V	R	V	V	Y	C	G	A	U	G	Y	K	S	K	Y	L
Monkey	M	A	L	A	V	R	V	V	Y	C	G	A	U	G	Y	K	S	K	Y	L
Sheep	M	A	V	A	V	R	V	V	Y	C	G	A	U	G	Y	K	P	K	Y	L
Pig	M	G	V	A	V	R	V	V	Y	C	G	A	U	G	Y	K	S	K	Y	L
Zebrafish	M	T	V	K	V	H	V	V	Y	C	G	G	U	G	Y	R	P	K	F	L
Chicken	**M**	**P**	**L**	**R**	**V**	**T**	**V**	**L**	**Y**	**C**	**G**	**A**	**U**	**G**	**Y**	**K**	**P**	**K**	**Y**	**E**
Mouse	Q	L	K	E	K	L	E	H	E	F	P	G	C	L	D	I	C	G	E	G
Rat	Q	L	K	E	K	L	E	H	E	F	P	G	C	L	D	I	C	G	E	G
Human	Q	L	K	K	K	L	E	D	E	F	P	G	R	L	D	I	C	G	E	G
Monkey	Q	L	K	K	K	L	E	D	E	F	P	G	R	L	D	I	C	G	E	G
Sheep	Q	L	K	K	K	L	E	D	E	F	P	S	R	L	D	I	C	G	E	G
Pig	Q	L	K	K	K	L	E	D	E	F	P	G	R	L	D	I	C	G	E	G
Zebrafish	K	L	K	T	L	L	E	D	E	F	P	N	E	L	E	I	T	G	E	G
Chicken	**R**	**L**	**R**	**A**	**E**	**L**	**E**	**K**	**R**	**F**	**P**	**G**	**A**	**L**	**E**	**M**	**R**	**G**	**Q**	**G**
Mouse	T	P	Q	V	T	G	F	F	E	V	T	V	A	G	K	L	V	H	S	K
Rat	T	P	Q	V	T	G	F	F	E	V	T	V	A	G	K	L	V	H	S	K
Human	T	P	Q	A	T	G	F	F	E	V	M	V	A	G	K	L	I	H	S	K
Monkey	T	P	Q	A	T	G	F	F	E	V	M	V	A	G	K	L	I	H	S	K
Sheep	T	P	Q	V	T	G	F	F	E	V	F	V	A	G	K	L	V	H	S	K
Pig	T	P	Q	V	T	G	F	F	E	V	L	V	A	G	K	L	V	H	S	K
Zebrafish	T	P	S	T	T	G	W	L	E	V	E	V	N	G	K	L	V	H	S	K
Chicken	**T**	**Q**	**E**	**V**	**T**	**G**	**W**	**F**	**E**	**V**	**T**	**V**	**G**	**S**	**R**	**L**	**V**	**H**	**S**	**K**
Mouse	K	R	G	D	G	Y	V	D	T	E	S	K	F	R	K	L	V	T	A	I
Rat	K	R	G	D	G	Y	V	D	T	E	S	K	F	R	K	L	V	T	A	I
Human	K	K	G	D	G	Y	V	D	T	E	S	K	F	L	K	L	V	A	A	I
Monkey	K	K	G	D	G	Y	V	D	T	E	S	K	F	L	K	L	V	A	A	I
Sheep	K	G	G	D	G	Y	V	D	T	E	S	K	F	L	K	L	V	A	A	I
Pig	K	G	G	D	G	Y	V	D	T	E	S	K	F	L	K	L	V	A	A	I
Zebrafish	K	N	G	D	G	F	V	D	S	D	S	K	M	Q	K	I	V	T	A	I
Chicken	**K**	**N**	**G**	**D**	**G**	**F**	**V**	**D**	**T**	**N**	**A**	**K**	**L**	**Q**	**R**	**I**	**V**	**A**	**A**	**I**
Mouse	K	A	A	L	A	Q	C	Q												
Rat	K	A	A	L	A	Q	C	Q												
Human	K	A	A	L	A	Q	G	-----												
Monkey	K	A	A	L	A	Q	G	-----												
Sheep	K	A	A	L	A	Q	A	-----												
Pig	K	A	A	L	A	Q	G	-----												
Zebrafish	E	Q	A	M	G	K	-----	-----												
Chicken	**Q**	**A**	**A**	**L**	**P**	-----	-----	-----												

Fig. 4 The deduced SeW amino acid sequences of mouse, rat, human, monkey, sheep, pig, zebrafish and chicken. Amino acid residue is shown by the one-letter code. The amino acid sequence of chicken selenoprotein W was deduced from the nucleotide sequence of the coding region and is shown in *bold*

Table 1 Sequence (seq) Homology of SeW cDNA and deduced amino acid (AA) of chicken, mouse, rat, human, monkey, sheep, pig and zebrafish

cDNA seq (AA seq)	*Mus musculus*	*Rattus norvegicus*	*Homo sapiens*	*Macaca mulatta*	*Ovis aries*	*Sus scrofa*	*Danio rerio*
Gallus gallus	59% (61%)	59% (61%)	59% (58%)	59% (58%)	59% (50%)	59% (59%)	23% (55%)
Mus musculus	100% (100%)	97% (100%)	85% (86%)	85% (86%)	82% (86%)	84% (86%)	59% (59%)
Rattus norvegicus	97% (100%)	100% (100%)	87% (86%)	86% (86%)	83% (86%)	85% (86%)	58% (59%)
Homo sapiens	85% (86%)	87% (86%)	100% (100%)	98% (100%)	89% (90%)	90% (93%)	51% (57%)
Macaca mulatta	85% (86%)	86% (86%)	98% (100%)	100% (100%)	89% (90%)	90% (93%)	50% (57%)
Ovis aries	82% (86%)	83% (86%)	89% (90%)	89% (90%)	100% (100%)	89% (91%)	49% (62%)
Sus scrofa	84% (86%)	85% (86%)	90% (93%)	90% (93%)	89% (91%)	100% (100%)	51% (59%)

Parenthesis indicates the homology of AA sequences between two different species

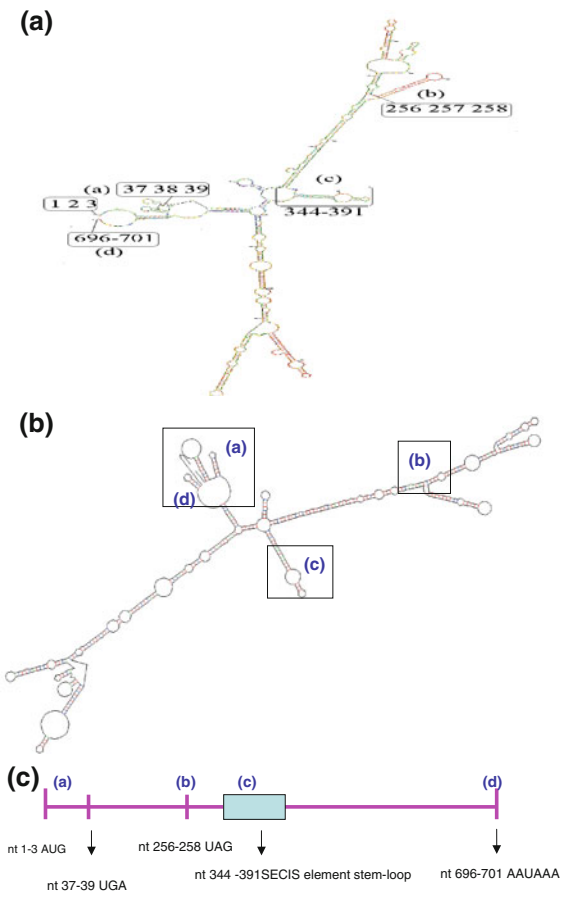

Fig. 5 The structure of chicken SeW mRNA. The secondary structure was predicted by using mfold web server (**a**) (Mathews et al. 1999; Zuker 2003) or RNAdraw (**b**) (Matzura and Wennborg 1996). The primary structure of chicken SeW mRNA (**c**) including start codon (AUG), selenocysteine (UGA), stop codon (UAG), SECIS element (nt 344-391) and polyadenylation signal (nt 696-701) are shown

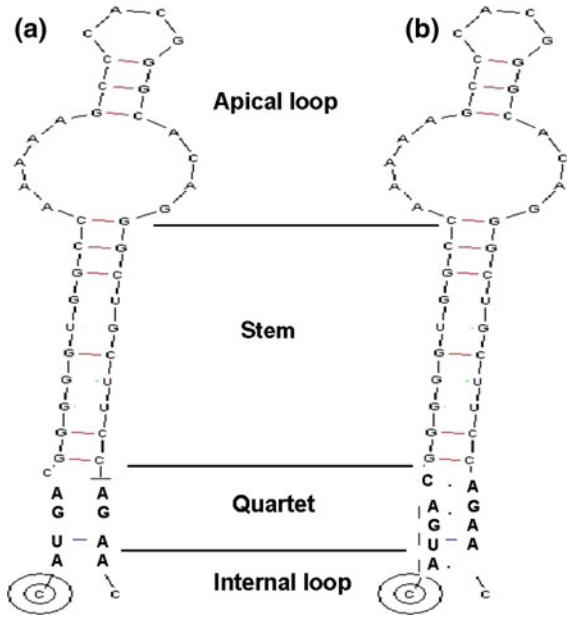

Fig. 6 Putative SECIS element stem-loop structure of chicken SeW mRNA. *Circled ring operator* indicates 5′-end. (**a**) G*G pair model (Low and Berry 1996) (**b**) G*A pair model (Walczak et al. 1996)

invariant adenosines within the apical bulge (Fig. 6). The models for G–G pairs (Fig. 6a) and G–A pairs (Fig. 6b) of the quartet are also proposed (Low and Berry 1996; Walczak et al. 1996).

Tissue distribution of SeW mRNA in chicken

The mRNA expression in various chicken tissues (heart, skeletal muscle, brain, testis, spleen, kidney,

lung, liver, stomach and intestine) was performed by RT-PCR using specific primers for chicken SeW. The results indicated that the mRNA expression was detected in skeletal muscle and brain of the chickens fed the commercial maized-based basal diet only (Fig. 7a) and the skeletal muscle expressed higher levels of chicken SeW mRNA than did the brain. However, after feeding the basal diet supplemented with 4 mg Se/kg diet as sodium selenite for 10 days, chicken SeW mRNA expression was detected in all tissues investigated (Fig. 7b). The β-actin mRNA shown in Fig. 7 was used as the internal control.

Discussion

SeW is mainly a cytosolic protein and its protein level is high in skeletal muscle, spleen, testis and brain of rat with the highest level in skeletal muscle (Yeh et al. 1995). The SeW mRNA expression was found to be high in chicken skeletal muscle in this study. Except for brain, the SeW mRNA expressions in other tissues of chickens fed commercial maize-based diet were too low to observe. Dietary supplementation with Se is the major source of Se for poultry. The adequate Se supplementation in complete feed for poultry is 0.3 mg Se/kg diet (Fischer et al. 2008), thus the low selenium content of 0.116 mg Se/kg diet in the commercial basal diet used in this study may account for the low expression of chicken SeW mRNA in most of the tissues. Although the Se content in the basal diet is low, the skeletal muscle and brain seem to be able to conserve selenium during Se deficiency in the chicken. Dietary selenium of 4 mg/kg diet up-regulated the expression of chicken SeW mRNA in all tissues investigated following 10 days of supplementation. This result is in accordance with previous findings that dietary Se regulates SeW expression in rat and sheep (Vendeland et al. 1995; Yeh et al. 1997a, b) and other selenoprotein expression in poultry (Sunde and Hadley 2010). The results from this study indicate that the expression of the SeW gene in various chicken tissues is regulated by dietary selenium supplementation, and the skeletal muscle is the most responsive tissue when dietary Se content is low. Among all tissues investigated in different species, the skeletal muscle is the only tissue where SeW is found to express with relatively abundant amounts across various species, including chicken in this study (Yeh et al. 1995, 1997a; Gu et al. 2000; Bellingham et al. 2003). These results imply that the function of SeW is strongly related to skeletal muscle metabolism and/or regulation. The results from a recent study investigating depletion of SeW in skeletal muscle cells in vitro had indicated that SeW is important for antioxidation in muscle cells (Wang et al. 2010).

The SeW mRNAs in mouse, rat, sheep and zebrafish use UGA as the stop codon and as the selenocysteine codon, while UAA is used as the stop codon in human, monkey and pig (Gu et al. 1997). A different stop codon, UAG, is used in chicken SeW mRNA. The selenocysteine (Sec) residue in chicken SeW is located at the 13th position, as all SeWs in other species are. The SeW protein sequence contains 87 amino acids in human, monkey, sheep and pig, and 88 amino acids in mouse and rat (Gu et al. 1997). The protein sequence of chicken SeW contains 85 aminio acids and appears to be shorter by 1–3 amino acid residues than other species including human, monkey, mouse, rat, sheep, pig and zebrafish (Whanger, 2009). The 23–59% homology was found in SeW cDNA sequences between chicken and other species with the lowest homology of 23% between chicken and zebra fish and the consistant homology of 59% between chicken and other mammals. The homology in SeW deduced amino acid sequences between chicken and other species was found to be 50–61%. The secondary structures of SeW SECIS elements found in various species including human, monkey, mouse, rat and sheep (Gu et al. 1997), as well as chicken in this study, are type II SECIS elements with invariant adenosines within the unpaired apical bulge.

Oxidative stress induces cellular regulations of antioxidative enzymes and influences cellular glutathione (GSH) level (Reed 1990). GSH is an important antioxidant in living organisms and the elevated GSH level exerts a protective effect in counteracting an oxidative stress in animals and humans (Meister 1991; Han et al. 1996; Ito et al. 1998). GSH was found to bind tightly to rat SeW, and the Cys_{37} residue in SeW from monkey muscle was identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) to be the GSH binding site (Beilstein et al. 1996; Gu et al. 1999). The Cys_{37} residue of SeWs in human, monkey, mouse, rat,

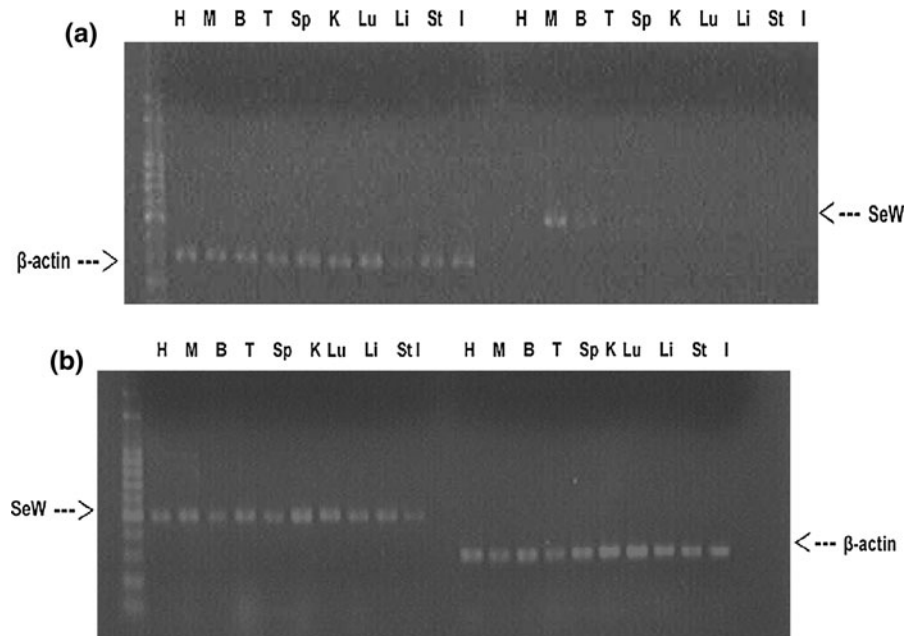

Fig. 7 Tissue distribution of chicken SeW mRNA fed a commercial diet without selenium supplementation (**a**) or with 4 mg Se/kg diet supplementation as sodium selenite (**b**) for 10 days. H: heart; M: skeletal muscle; B: brain; T: testis; Sp: spleen; K: kidney; Lu: lung; Li: liver; St: stomach and I: intestine

sheep and pig is not conserved in chicken and zebrafish SeWs (Whanger 2009). The Cys_9 and Sec_{13} are the conserved amino acids in SeWs from all species (human, monkey, mouse, rat, sheep, pig, zebrafish and chicken) identified thus far. Based on the previous finding, Cys_9 gave a different analytical elution pattern from Cys_{37}, and there is possibility that GSH may form a bridge between the sulfur atom on Cys_9 and the selenium atom on Sec_{13} (Beilstein et al. 1996). Due to the characteristic similarity to the sulfur atom, Sec is able to catalyze oxidation of thiols in the reducing environment of the cytoplasm (Hawkes and Alkan 2010). Thus, SeW from different species may have GSH binding to different amino acids or may not have GSH binding to this protein. This possibility may imply the differential role and biological function that SeW plays in different species. The previous report from selective production of rat mutant selenoprotein W indicated that two different forms of rat mutant SeW were generated. Under aerobic conditions, SeW production without GSH binding is favored, whereas GSH-bound SeW is preferred under anaerobic conditions (Bauman et al. 2004). The GSH redox cycle is one of the important detoxification pathways in vivo (Reed 1990), thus, the redox status in different oxidative conditions may affect the binding of GSH to SeW. Further investigation of the structural and functional roles GSH plays in SeW is needed to clarify the function of this selenoprotein.

In summary, chicken SeW contains one well-conserved selenocysteine (Sec) residue at the 13th position encoded by the UGA codon and the SECIS element of chicken SeW mRNA is type II with invariant adenosines within the apical bulge. The chicken SeW mRNA expression is high in skeletal muscle followed by brain. The dietary selenium supplementation up-regulates SeW gene expression in the chicken and the skeletal muscle is the most responsive tissue when dietary Se content is low.

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