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Characterization of Selenoprotein M and Its Response to Selenium Deficiency in Chicken Brain

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Abstract Selenoprotein M (SelM) may function as thiol disulfide oxidoreductase that participates in the formation of disulfide bonds and can be implicated in calcium responses. SelM may have a functional role in catalyzing free radicals and has been associated with Alzheimer's disease (AD). However, studies of SelM in chicken remain very limited. In this study, two groups of day-old broiler chicks (n = 40/group) were fed a corn-soy basal diet (BD, 13 μg Se/kg) and BD supplemented with Se (as sodium selenite) at 0.3 mg/kg. The brain was collected at 14, 21, 28, and 42 days of age. We performed a sequence analysis and predicted the structure and function of SelM. We also investigated the effects of Se deficiency on the expression of *Selt*, *Selw*, and *Selm* and the Se status in the chicken brain. The results show that Se deficiency induced the lower ($P < 0.05$) Se content, glutathione peroxidase (GPx), and catalase (CAT) activities; increased ($P < 0.05$) malondialdehyde (MDA) content; and reduced ($P < 0.05$) the expression of *Selm* messenger RNA (mRNA) and protein abundance of SelM in the brain. However, there were no significant brain *Selt* and *Selw* mRNA levels by dietary Se deficiency in chicks. The different regulations of these three redox (Rdx) protein expressions by Se deficiency represent a novel finding of the present study. Our results demonstrated that SelM may have an important role in protecting against oxidative damage in the brain of chicken, which might shed light on the role of SelM in human neurodegenerative disease. More studies are needed to confirm our conclusion.

Keywords Brain · Chick · Selenocysteine · Selenoprotein M · Selenium

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Abbreviations

AD	Alzheimer's disease
Se	Selenium
SECIS	Selenocysteine insertion sequence
SelT	Selenoprotein T
SelM	Selenoprotein M
SelW	Selenoprotein W

Introduction

Selenium (Se), an essential trace element for many living organisms, can assist cells to resist oxidative damage. Se in vivo is primary present as various selenoproteins to maintain the balance of the cellular redox state [1]. Selenoproteins are defined by incorporation of Se into amino acid selenocysteine. This human and avian family all consist 25 selenoproteins [2–4], which play important roles in redox regulation, detoxification, immune system protection, and viral suppression [5–7]. However, the biological functions of some selenoproteins identified recently remain largely unknown, including some redox (Rdx) proteins containing a diselenide bond in a common redox motif [8]. This motif that consist of CxxU (U designates selenocysteine) has been identified in a

subset of selenoproteins, including selenoprotein M (SelM) [9], selenoprotein W (SelW) [10, 11], and selenoprotein T (SelT) [12]. It has been proposed that these Rdx proteins exhibit a thioredoxin-like fold and a conserved CxxU motif, suggesting a redox function. SelW, the first discovered selenoprotein, is the well-characterized protein. SelW is particularly abundant in muscle, and its deficiency has been implicated in white muscle disease [13, 14]. SelT is a protein involved in intracellular Ca^{2+} mobilization and neuroendocrine secretion, and SelT is largely regulated by pituitary adenylate cyclase-activating polypeptide (PACAP) gene [15]. Se and selenoproteins have been suggested to implicate in neurodegenerative disease, such as Alzheimer's disease (AD) and Parkinson's disease [16].

SelM was first reported as a new selenoprotein using bioinformatics methods [9, 17]. It is highly conserved from plants to humans and localizes to the endoplasmic reticulum. In pig, SelM is ubiquitously expressed in many tissues, including muscle, liver, kidney, cerebral cortex, pituitary, thyroid, and testis. In human, it is expressed in many tissues with the highest levels in the brain [9], supporting its possible function in AD. Although the distribution, function, and structure of SelM in mammals have been well studied, studies of SelM in chicken remain very limited. In this study, we aim to analyze the nucleotide and amino acid sequences of chicken SelM. We also compared the Se content; glutathione peroxidase (GPx) activity; catalase (CAT) activity; malondialdehyde (MDA) content; messenger RNA (mRNA) level of *Selt*, *Selw*, and *Selm*; and protein level of SelM in the brain between Se-deficient and Se groups.

Materials and Methods

Animal, Diet, and Experimental Design

All procedures in this experiment were approved by the Institutional Animal Care and Use Committee of China Agricultural University. A total of 80 day-old male broiler poults (Dafa Zhengda Poultry Co., Ltd., Beijing, China) were randomly divided into two groups. One group was treated with basal diet composed of corn and soybean that is obtained from Se-deficient area (Sichuan, China) (Se-deficient group) (n = 40), and the other group was treated with Se basal diet with sodium selenite at 0.3 mg/kg (Sigma-Aldrich, St. Louis, MO, USA) (Se group) (n = 40). The Se concentrations were 13 and 310 μg/kg in Se-deficient and Se groups, respectively. Food and water were provided ad libitum. All procedures, as well as the care, housing, and handling of the animals, were conducted according to accepted commercial management practices. On days 14, 21, 28, and 42, chickens (n = 10/group) were anesthetized with sodium pentobarbital and brain was collected and frozen in liquid nitrogen for analysis.

Selm Sequence Analysis, Selenocysteine Insertion Sequence Element Prediction, 3D Molecular Modeling, and Phylogenetic Analysis

With selenocysteine insertion sequence (SECIS), UGA codon can be translated into Sec, which has been regarded as a marker of selenoprotein mRNA. In order to confirm the novel RNA sequence and to identify the SECIS element, we used the SECISearch engine (http://seblastian.crg.es/, SECISearch3) [18] to analyze the primary sequence and secondary structure of chicken *Selm* mRNA. The complementary DNA (cDNA) sequence and deduced amino acid sequence were analyzed using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast) and the Expert Protein Analysis System (http://www.expasy.org/). The 3D structure and biological function of chicken SelM were predicted using the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) [19, 20]. The evolutionary and tree homology analysis was constructed using the DNAMAN 6 (Lynnon BioSoft, Canada) and MEGA 5 [21].

Biochemical Assays

Se concentrations in brains obtained from chickens were measured using the hydride generation-atomic fluorescence spectrometer (AFS-3200; Yongtuo Instruments) against the standard reference of Se (GBW(E)080441, National Research Center for Certified Reference Materials) [22]. Briefly, brain tissues were homogenized on ice in physiological saline and centrifuged at 1000×g and supernatants were collected for GPx, CAT, and MDA activity analysis using kits provided by Jiancheng Biotechnology Research Institute (Nanjing, China). GPx activity was measured spectrophotometrically at 37 °C at 412 nm as described previously [23]. The CAT assay was measured spectrophotometrically at 520 nm. The formation of MDA was determined as an indicator of lipid peroxidation measuring at 532 nm [24]. Protein concentration was determined using the method by Bradford [25].

Real-Time Quantitative PCR Analysis of *Selm*, *Selw*, and *Selt*

Literature search [26] allowed us to target on *Selm*, *Selw*, and *Selt* genes in chicks. To determine effects of dietary Se on the mRNA expression of these genes, we isolated total mRNA from the brain (50 to 100 mg tissue) of five most representative chicks from each group. The RNA sample preparation, quantitative PCR (Q-PCR) procedure, and the relative mRNA abundance qualification were the same as previously described by our group [4]. Primers (Table 1) for the three selenoprotein genes and two reference genes, β-actin gene (*Actb*) and glyceraldehyde 3-phosphate dehydrogenase gene

Table 1 Primers used for real-time quantitative PCR of *Selm*, *Selw*, *Selt*, *Actb*, and *Gapdh*

Gene	Number	Sequence
Selm	NM_001277859.1	5′-AAGAAGGACCACCCAGACCT-3′
		5′-GCTGTCCTGTCTCCCTCATC-3′
Selw	NM_001166327.1	5′-TGGTGTGGGTCTGCTTTACG-3′
		5′-CCAAAGCTGGAAGGTGCAA-3′
Selt	NP_001006557.3	5′-AGGAGTACATGCGGGTCATCA-3′
		5′-GACAGACAGGAAGGATGCTATGTG-3′
Actb	L08165.1	5′-ACCTGAGCGCAAGTACTCTGTCT-3′
		5′-CATCGTACTCCTGCTTGCTGAT-3′
Gapdh	NM_204305.1	5′-GGTGCTAAGCGTGTTATCATCTCA-3′
		5′-CATGGTTGACACCCATCACAA-3′

(*Gapdh*), were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA, USA).

Western blot Analysis

Tissues were homogenized with cell lysis buffer for western and IP (catalog no. P0013; Beyotime Institute of Biotechnology) and centrifuged at 12,000×*g* for 10 min at 4 °C. The supernatants (10~40 μg protein/lane) were loaded onto a SDS-PAGE gel (12.5 %), followed by transfer of proteins to polyvinylidene difluoride membranes (Millipore, Temecula, CA, USA). The membrane was incubated with primary anti-SelM antibody (1:500; Santa Cruz Biotech, Santa Cruz, CA, USA) and anti-β-actin (1:1000; Biosynthesis Biotechnology, Beijing, China), followed by incubation with

a

ATGCGGCGGGCGGCGCTGGCCGCGCTGCTGCTGCTGGCGGCGGCGGCGGGGATCGAGCGGCGGCCGCCCCGGGGCTTGGC 80
GAGGGGCAAAGTGGAGACGTGCGGTGGG**TGA**CGGCTGAGCCGGCTGCCGGAGGTGAAGGCCTTCGTCAGCCAGGACATC 160
CCGCTGTACCATAACCTGGAGATGAAGCACCTGCCCGGCGCCGACCCTGAGCTCGTGCTGCTTAGCTTCAGATACGAGGAG 240
CTGGAGAGAATCCCCCTGAGCGACATGACCCGGGAGGAGATCAACCAGCTGGTGCAGGAGCTGGGCTTCTACCGCAAGGA 320
GACTCCCGAAGCTCCTGTGCCCGAGGAGTTCCAGTTTGCCCCTGCCAAGCCACTGCCCACCCTAACACCCCGCAGGGCTCC 400
TGCAGCTGACGGCAAGACCCTGTCTGAACAGGACAAGAAGGACCACCCAGACCTG**TAA**tgagcagtgccaggcggtaggaggccctgcagg 492
cagtccttagagcgtgaggatgagggagacaggacagctgcctcttcacacggggttggggagcatgctgcggggcggcaagagtgtgagcaggaaggagagagatgttccctgctgctgtcactgt 619
tgcttggtgctctggagcctctgtcccaagggtgccctgaatggtgccagccttgagcagaggctcctgagcgctgcagacatctgagtgagcccatgtgcctgtcactgccttccccctgagccacgca 749
gcccagccgttctgacacgattagtgaggagaagcagagatctttttgctcctctcccatgccccaaggttcagccaactcctggtgctgGACAGCAGTATCCTACCTGCACAGGA 865
CAGG**ATGAA**ACTCTCAGCAG**AAA**GCTCCACATGCTGAGGGT**AGAT**GTGCTGAGTCCTGGAGGTGGGACAGCTgcctcccggga 948

b

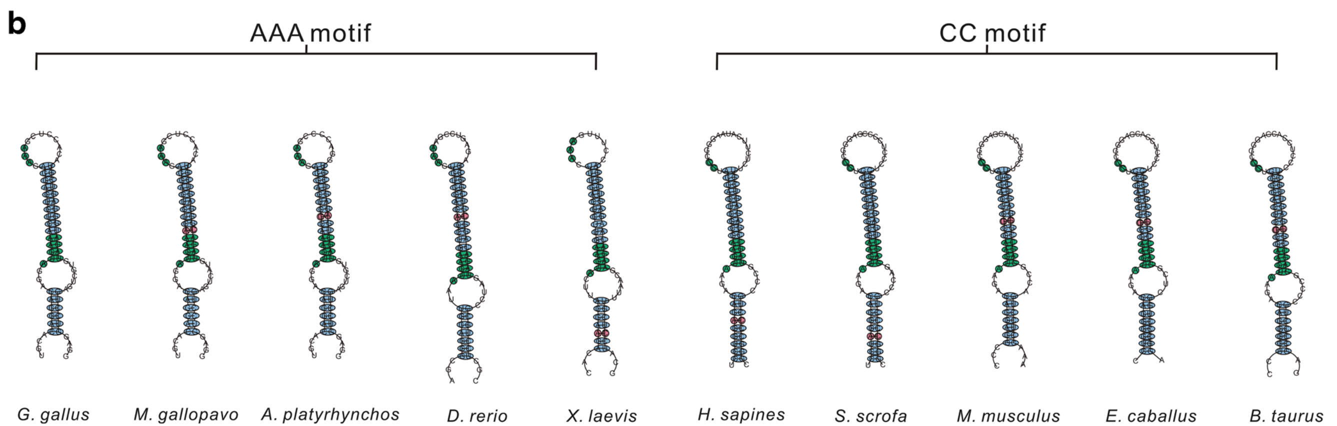
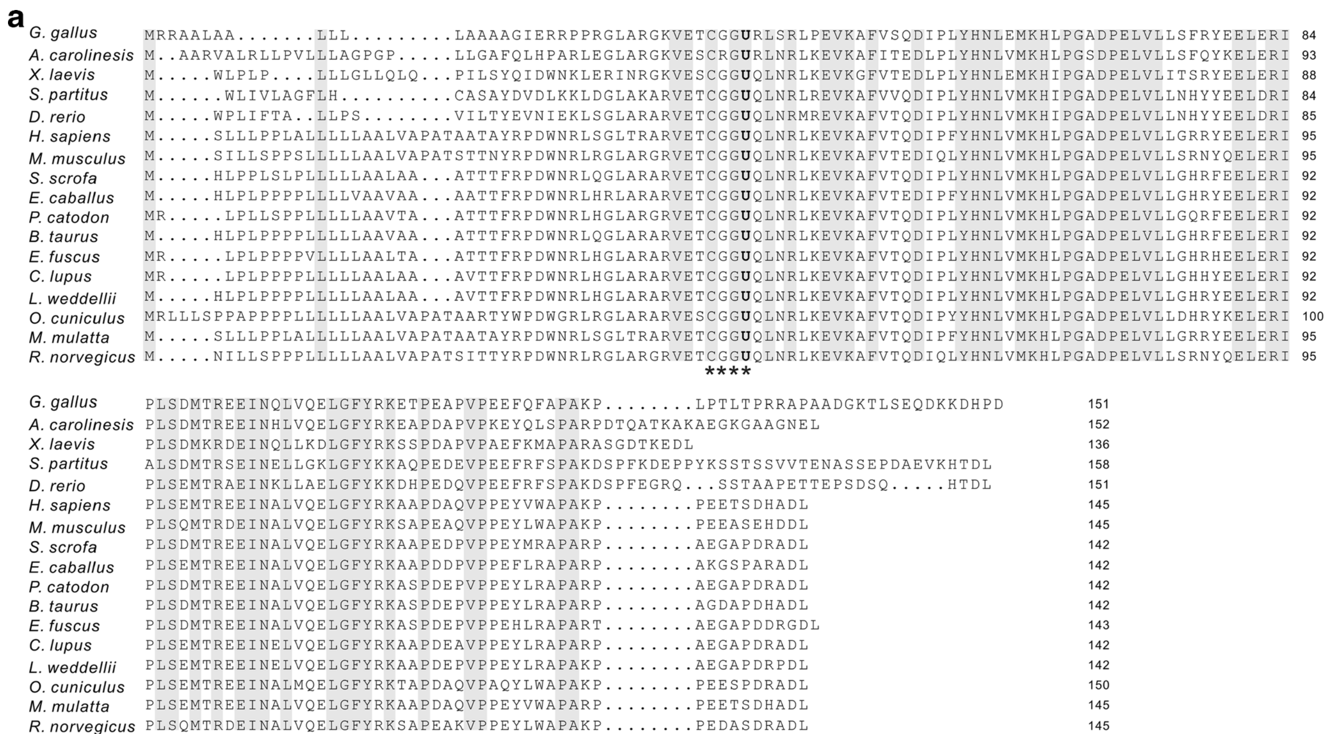


Fig. 1 Chicken *Selm* cDNA and its selenocysteine insertion sequence (SECIS). **a** The cDNA sequence of chicken *Selm*. The sequence in *uppercase* is the coding sequence from ATG to TAA (in *bold*), and the framed TGA at 37th triplet from the initiation codon encodes selenocysteine (Sec). *The underlined letters in bold* in the 3′-untranslated region indicate that the highly conserved nucleotides are in the SECIS. **b** The SECIS structures were predicted using the SECISearch program with appropriate patterns. The highly conserved nucleotides are in *bold*. Accession numbers of the sequences mentioned above are NM_001277859.1 (*G. gallus*), XM_003211094.1 (*M. gallopavo*), XM_005016259.1 (*A. platyrhynchos*), NM_178286.3 (*D. rerio*), NM_001092248.1 (*X. laevis*), NM_080430.2 (*H. sapiens*), NM_001161648.1 (*S. scrofa*), NM_053267.2 (*M. musculus*), NM_001168402.1 (*E. caballus*), and NM_001163171.1 (*B. taurus*)

horseradish peroxidase-conjugated secondary antibody (1:10,000; ZSGB Biotech, Beijing, China). The washed blot was developed using enhanced chemiluminescence reagent (Millipore) and exposed to X-ray films.

Statistical Analysis

Statistical analysis was performed using SPSS, version 13 (Chicago, IL, USA). Data were presented as mean ± SD.



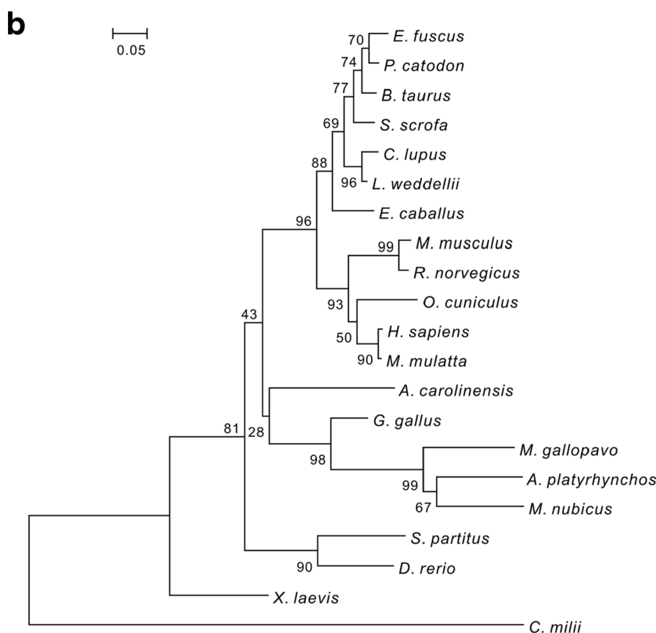


Fig. 2 Alignment and phylogeny analyses of SelM based on the amino acid sequences. **a** The conserved CXXU motif (CGGU in the chicken) is indicated by the *asterisks*. The *gray-colored letters* indicate conserved amino acid residue across the 17 species. **b** The phylogenetic analyses were conducted using MEGA5 and encompassed 20 SelM proteins. The bootstrap consensus tree generated using the neighbor-joining method with 500 replicates and pairwise deletion options was taken to represent the evolutionary history of the 20 proteins. The statistics and frequency are presented at each of the nodes, and *the length of the distance scale bar at the bottom of the panel* defines 0.05 of the genetic distance

Table 2 Sequence (seq) homology of SelM cDNA and deduced amino acid (AA) of chicken, mouse, rat, human, cow, duck, turkey, zebrafish, and frog

cDNA seq (AA seq)	*M. musculus*, % (%)	*R. norvegicus*, % (%)	*H. sapiens*, % (%)	*B. taurus*, % (%)	*A. platyrhynchos*, % (%)	*M. gallopavo*, % (%)	*D. rerio*, % (%)	*X. laevis*, % (%)
G. gallus	76 (79)	75 (80)	78 (79)	82 (79)	78 (84)	94 (97)	73 (58)	73 (73)
M. musculus	100 (100)	93 (94)	80 (79)	75 (79)	74 (75)	76 (79)	76 (65)	72 (66)
R. norvegicus	93 (94)	100 (100)	80 (86)	76 (82)	76 (75)	75 (79)	74 (66)	71 (65)
H. sapiens	80 (79)	80 (86)	100 (100)	81 (84)	79 (81)	77 (81)	76 (68)	73 (65)
B. taurus	75 (79)	76 (82)	81 (84)	100 (100)	78 (81)	82 (79)	74 (64)	74 (67)
A.platyrhynchos	74 (75)	76 (75)	79 (81)	78 (81)	100 (100)	75 (84)	72 (60)	75 (76)
M. gallopavo	76 (79)	75 (79)	77 (81)	82 (79)	75 (84)	100 (100)	72 (71)	72 (76)
D. rerio	76 (65)	74 (66)	76 (68)	74 (64)	72 (60)	72 (71)	100 (100)	74 (59)

Parenthesis indicates the homology of AA sequences between the two different species

Difference between groups was determined by one-way ANOVA using a significance level of $P < 0.05$. The data were assessed using the Tukey-Kramer method for multiple comparisons.

Results

Sequence and Structure of Chicken *Selm*

The characteristic of chicken *Selm* cDNA and its SECIS element is illustrated in Fig. 1. The *Selm* coding sequence (CDS) had 459 bases with an in-frame TGA triplet. The sequence theoretically encoded 152 amino acid residues, and the Sec was the 37th residue (Fig. 1a). The chicken *Selm* had 78 % CDS homology to human *Selm*. According to the generally used classification method [27] and SECISearch program analysis, a SECIS element, with the conserved denosine (–AAA–) rather than the cytidine (–CC–) motif in the apical loop was found in the 3′-untranslated region of the cDNA (mRNA), which is similar to the majority of avian species. Figure 1b compares the *Selm* SECIS elements in chick with other species, finding that the SECIS element had the –AAA– motif in *Gallus gallus* (chick), *Meleagris gallopavo* (turkey), *Anas platyrhynchos* (duck), *Danio rerio* (zebrafish), and *Xenopus tropicalis* (frog). In contrast, the SECIS element had the –CC– motif in *Homo sapiens* (human), *Sus scrofa* (pig), *Mus musculus* (mouse), *Equus caballus* (horse), and *Bos taurus* (cattle).

Homology and Phylogeny Analyses

The chicken SelM protein was aligned with 16 species (Fig. 2a), including *Anolis carolinensis* (green anole), *X. tropicalis* (frog), *Stegastes partitus* (bicolor damselfish), *D. rerio* (zebrafish), *H. sapiens* (human), *M. musculus* (mouse), *S. scrofa* (pig), *E. caballus* (horse), *Physeter catodon* (sperm whale), *B. taurus* (cattle), *Eptesicus fuscus* (bat), *Canis lupus* (dog), *Leptonychotes weddellii* (Weddell seal), *Oryctolagus cuniculus* (rabbit), *Macaca mulatta* (monkey), and *Rattus norvegicus* (rat). It shows an overall 58 % identity with a highly conserved thioredoxin-like domain containing a CXXU motif across all the species.

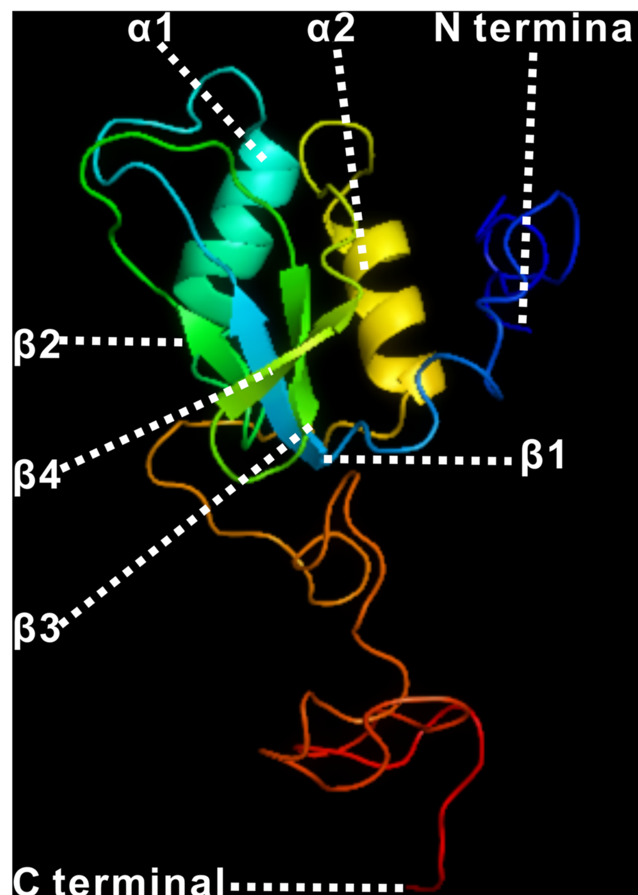


Fig. 3 The 3D structure of chicken SelM. α and β indicate α-helix and β-sheet, respectively. The active Sec site residue is located in the loop between β_1 and α_1 in a pocket on the protein surface

The amino acid sequence of chicken SelM shares a 79, 84, 97, and 58 % identity with SelM, in humans, duck, turkey, and zebrafish, respectively (Table 2). The nucleotide identity range of CDS of *Selm* is 72 to 94 % between chicken and other animals, including mammals and aquatic invertebrates (Table 2). In phylogeny (Fig. 2b), when SelM from the 20 species was genetically clustered, the Phasianinae, chicken, and turkey had the shortest distance.

The Predicted 3D Structure of SelM

Figure 3 shows that chicken SelM consists of a two stranded α-helices with four extended β-sheets. The molecule is characterized by a β_1 (25–31)-α_1(38–54)-β_2(58–63)-β_3(69–73)-β_4(79–85)-α_2(115–127) secondary structure pattern, wherein β_1 and β_2 are parallel strands forming one classical β-α-β motif. The predicted active Sec (U) site is located in the loop (residues 37) between β_1 and α_1.

Se Content, GPx, and CAT Activities and MDA Content in Brain Tissue

In brains collected at each time point, Se level was significantly lower in Se-deficient group than in Se group ($P < 0.05$) (Fig. 4(a)). In the Se group, compared with Se level in brains collected at 14 and 21 days, Se level at 28 and 42 days was decreased ($P < 0.05$). In Se-deficient group, GPx activity in the brain was significantly lower than that in Se group collected at different time points ($P < 0.05$) (Fig. 4(b)). In the Se-deficient group, GPx activity was decreased in the sampling time point from 14 to 42 days ($P < 0.05$). However, there was no significant difference in GPx activity at different time points in the Se group. In the Se-deficient group, CAT activity in the brain was significantly lower than that in the Se group brain collected at different time points ($P < 0.05$) (Fig. 4(c)). In the Se-deficient group, CAT activity was decreased in the sampling time point from 14 to 42 days ($P < 0.05$). However, there was no significant difference in CAT activity at different

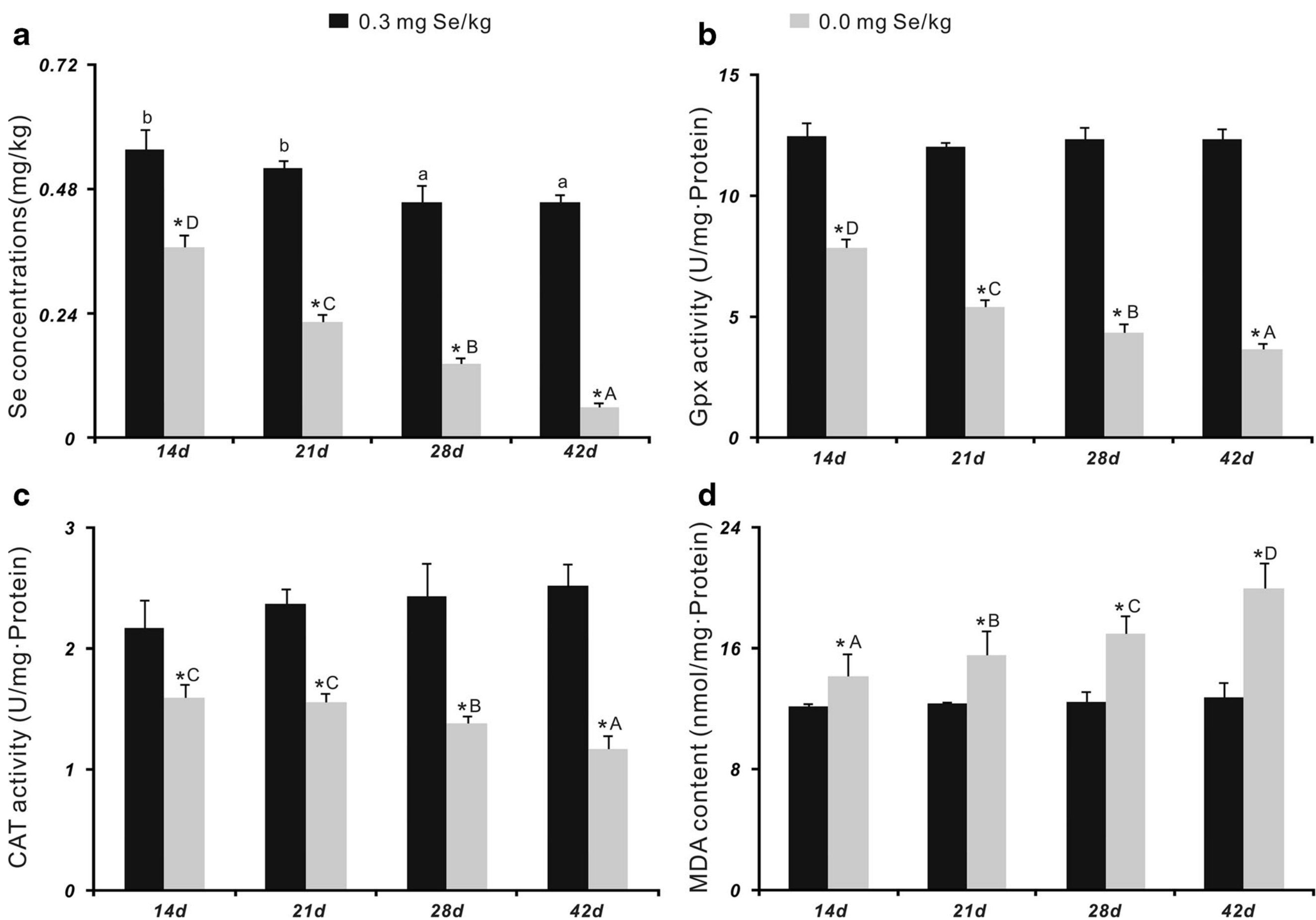


Fig. 4 Effects of dietary Se concentration on Se content (*a*), GPx activity (*b*), CAT activity (*c*), and MDA content (*d*) in the brain of chickens fed a basal selenium-deficient diet (BD) plus 0.0 and 3.0 mg Se/kg in four sampling points (*d* = days). Data are presented as means ± standard error of the mean (SEM), $n = 4$ (*a*) or $n = 5$ (*b*–*d*). Within the same sampling points, *asterisk* ($P < 0.05$) shows differences under different dietary Se concentrations. At the same concentrations of dietary Se, *bars with different letters* (*capital letter* in 0.0 mg Se/kg group and *small letter* in 0.3 mg Se/kg group) are statistically significantly different at each time point by one-way analysis of variance followed by Tukey-Kramer multiple comparison test ($P < 0.05$)

time points in the Se group. In the Se-deficient group, MDA content in the brain was significantly greater than that in the Se group brain collected at different time points ($P < 0.05$) (Fig. 4(d)). In the Se-deficient group, MDA content was increased in the sampling time point from 14 to 42 days ($P < 0.05$). However, there was no significant difference in MDA content at different time points in the Se group.

The mRNA Level of *Selt*, *Selw*, and *Selm* and Protein Expression of SelM in Brain

Figure 5(a) shows that mRNA levels of *Selt* in the brain collected at all time points are non-comparable between the two groups. The mRNA level of *Selw* in the brain of Se-deficient group collected at 42 days was significantly lower than in the brain collected from the Se group ($P < 0.05$). No significant difference in *Selw* level was detected among the other time points (Fig. 5(b)). Figure 6(a) shows that mRNA levels of *Selm* in the brain collected from the Se-deficient group were significantly lower than those in the brain from the Se group collected at all time points ($P < 0.05$). We also examined the protein expression of SelM in brains, finding that the SelM protein in the Se-deficient group was significantly lower than that in Se group collected at 14 days (Fig. 6(b)), 21 days (Fig. 6(c)), 28 days (Fig. 6(d)), and 42 days (Fig. 6(e)) ($P < 0.05$).

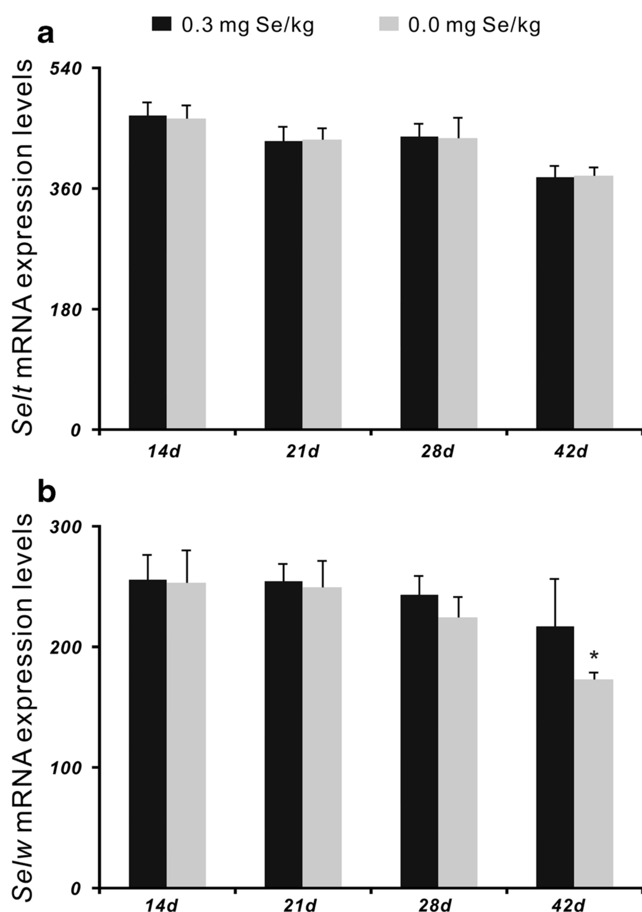


Fig. 5 Effects of dietary Se concentration on *Selt* mRNA abundance (*a*) and *Selw* mRNA abundance (*b*) in the brain of chickens fed a basal selenium-deficient diet (BD) plus 0.0 and 3.0 mg Se/kg in four sampling points (*d* = days). Data are presented as means ± standard error of the mean (SEM), $n = 5$. Within the same sampling points, *asterisk* ($P < 0.05$) shows differences under different dietary Se concentrations

Discussion

In our study, we found that chicken SelM contains one well-conserved Sec. The chicken SelM protein is characterized by a β-α-β-β-β-α secondary structure pattern that forms a classical motif observed in thioredoxin-like fold proteins. In the Se-deficient group, GPx and CAT activities were decreased in the sampling time point. In contrast, the MDA content was increased by Se deficiency at all time points. Meanwhile, the mRNA and protein levels of *Selm* in the brain of Se-deficient group were significantly lower than those in the brain of Se group collected at all time points [28]. To the best of our knowledge, this is the first work that reports the structural and functional predictions of SelM in chicken.

Consistent with the *Selm* SECIS elements in other species (i.e., turkey, duck, zebrafish, and frog), the chicken *Selm* SECIS elements also contains –AAA– sequence but not –CC– in the apical loop. However, the substitution of adenosines (A) for cytidines (C) shows no observable effect on Sec insertion into SelM. Pervious study has demonstrated that the SECIS element with –CC– motif appears to be universal in the *Selm* genes of mammalian animals [9]. In order to understand the evolutionary meaning of forms and motifs in the *Selm* SECIS element, we did a phylogenetic analysis encompassing a greater number of species. Our finding indicates that similar to its mammalian homolog, chicken SelM may play a role in redox regulation [17]. The observed highly homology of SelM indicates that chicken SelM was derived from a common ancestor. Our 3D structural analysis demonstrated that chicken SelM was similar to that of pig SelM [9], chicken SelW [10, 11], and chicken SelT [12], suggesting that they might exhibit similar functions.

In this study, we examined mRNA level of *Selm*, *Selw*, and *Selt* to assess the response of SelM to Se. In the Se-deficient group, we found decreased level of Se content, GPx and CAT activities, and *Selm* mRNA level in the brain compared with those in Se groups. However, no significant difference in *Selt* and *Selw* mRNA levels between Se-deficient and Se groups was observed. In contrast, the Se-deficient chicks had higher MDA content in the brain than the Se chicks at each time point. In general, antioxidant defense systems eliminate reactive oxygen species (ROS) or other radical species to prevent oxidative stress, which is defined as an alteration in the steady-state balance between oxidant and antioxidant agents. It has

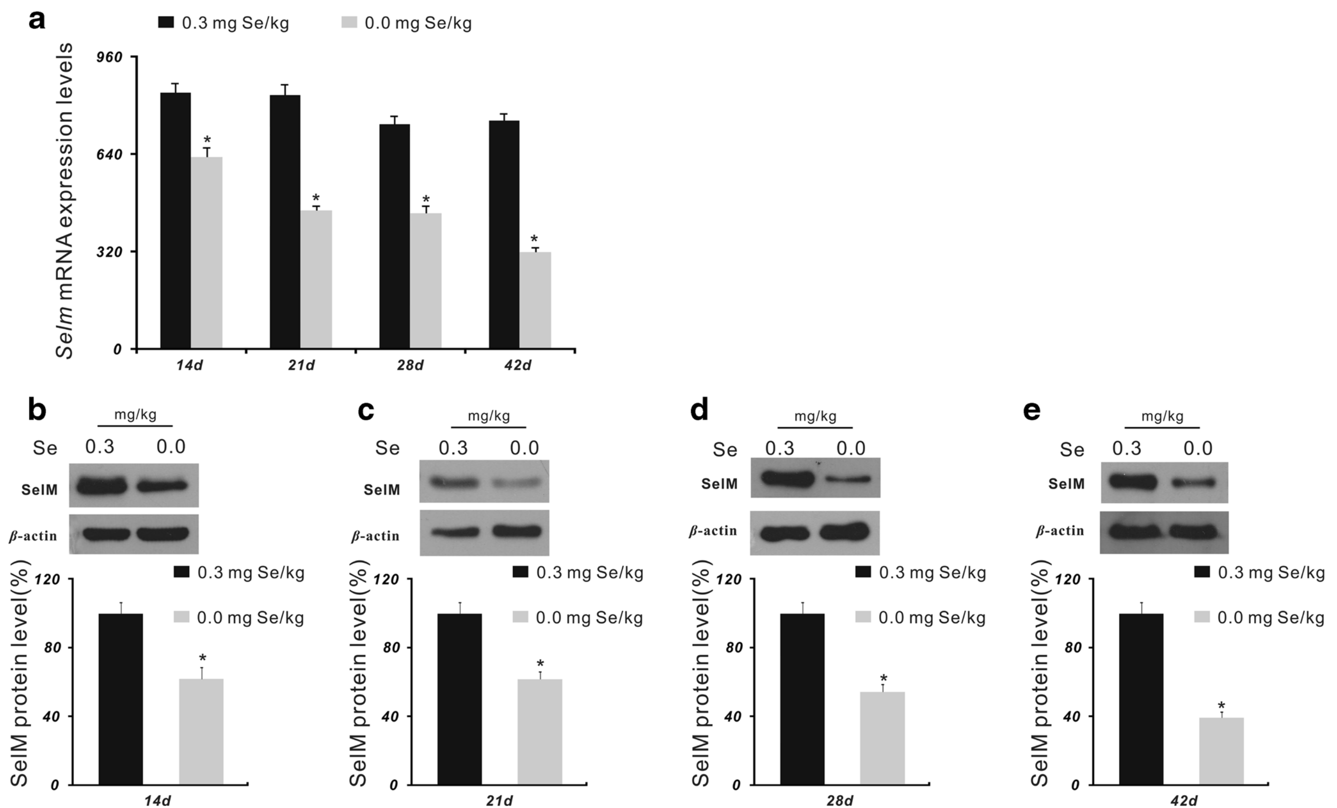


Fig. 6 Effects of dietary Se concentration on the *a* brain tissue *Selm* mRNA abundance and brain tissue protein level of SelM in 14 days (*b*), 21 days (*c*), 28 days (*d*), and 42 days (*e*) of chickens fed a basal selenium-deficient diet (BD) plus 0.0 and 3.0 mg Se/kg. Data are presented as means ± standard error of the mean (SEM), $n = 5$ (*a*) or $n = 4$ (*b*–*e*). Within the same sampling points, *asterisk* ($P < 0.05$) shows differences under different dietary Se concentrations

been shown that under Se-deficient status, the activity of some antioxidative enzymes, such as GPx and CAT, the first line of cellular defense against potential oxidative damage [29], might be inhibited in various tissues, such as liver and muscle in poults [4], plasma in mice [30], and liver and brain of rat [31]. Consistent with previous studies, we observed decreased GPx, CAT, and Se in the Se-deficient group. Se levels in the brain are not high compared with those in most other organs and remain remarkably stable even during Se deficiencies [32, 33]. The results indicated that Se may be critical for the maintenance of brain function and may first meet the Se demand of the brain at the expense of other organs. However, in chicken feed with low-Se diet for a long time, the brain is also influenced by Se deficiency.

Compared with the Se group, we observed similar mRNA level of *Selt* and *Selw*, decreased mRNA level of *Selm*, and decreased protein level of SelM in the Se-deficient group. In fact, SelM, SelT, and SelW are the three important Rdx proteins in selenoprotein family and are proposed to possess a thioredoxin-like fold and a conserved CxxC or CxxU motif with redox function [34]. Previous study has showed that SelT was expressed in neural progenitors in various regions, such as the cortex and cerebellum, but was undetectable in most adult nervous cells [35]. SelT expression was maintained at stable level in several adult endocrine tissues, such as pituitary, thyroid, and testis. SelW was widespread in neurons and neuropil of mouse brain and appeared in soma and processes of neurons in culture [36]. Pyramidal neurons of the cortex and hippocampus express high levels of SelW. In our study, we did not observe significant difference in *Selt* and *Selw* mRNA level (14, 21, and 28 days), indicating the stable Se dietary-independent expression pattern. Se deficiency may cause irreversible changes in the neuronal cells and brain injury [37]. Clinical studies have revealed that Se deficiency is associated with various neuro diseases, such as cognitive impairment, seizures, Parkinson's disease, and Alzheimer disease [38]. Taken together with the observed decreased Selm mRNA level in our study, we speculated that SelM is one of the major selenoperoxidases contributing to the prevention of brain injury possibly through the peroxide scavenging and antioxidant functions. Compared with other avian selenoproteins [4, 28, 39], in the present study, SelM was more sensitive to Se deficiency in the brain. This might indicate that SelM could be a molecular biomarker that predicts Se status in the brain.

In conclusion, the sequence of SelM in chicken is similar to that in other species. In the Se-deficient group, GPx and CAT activities and mRNA level of *Selm* were decreased

significantly than those in the Se group. Our results demonstrated that SelM may have an important role in protecting against oxidative damage in the brain, which might shed light on the role of SelM in neurodegenerative disease in human. More studies in other species are needed to confirm our conclusion.

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Conflict of Interest The authors declare that they have no competing interests.

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