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FOR THE RECORD

Selenoproteinless animals: Selenophosphate synthetase SPS1 functions in a pathway unrelated to selenocysteine biosynthesis

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Abstract

Proteins containing the 21st amino acid, selenocysteine (Sec), have been described in all three domains of life, but the composition of selenoproteomes in organisms varies significantly. Here, we report that aquatic arthropods possess many selenoproteins also detected in other animals and unicellular eukaryotes, and that most of these proteins were either lost or replaced with cysteine-containing homologs in insects. As a result of this selective selenoproteome reduction, fruit flies and mosquitoes have three known selenoproteins, and the honeybee, *Apis mellifera*, a single detected candidate selenoprotein. Moreover, we identified the red flour beetle, *Tribolium castaneum*, and the silkworm, *Bombyx mori*, as the first animals that lack any Sec-containing proteins. These insects also lost the Sec biosynthesis and insertion machinery, but selenophosphate synthetase 1 (SPS1), an enzyme previously implicated in Sec biosynthesis, is present in all insects, including *T. castaneum* and *B. mori*. These data indicate that SPS1 functions in a pathway unrelated to selenoprotein synthesis. Since SPS1 evolved from a protein that utilizes selenium for Sec biosynthesis, an attractive possibility is that SPS1 may define a new pathway of selenium utilization in animals.

Keywords: selenophosphate synthetase; selenocysteine; selenoproteome; Sec-insertion machinery

Selenocysteine (Sec), the 21st naturally occurring protein amino acid, is inserted into nascent polypeptides at UGA codons. The use of Sec instead of cysteine (Cys) often results in a higher enzyme activity, providing a competitive advantage to the organisms that utilize it (Kim and Gladyshev 2005). In eukaryotes, the number of proteins containing Sec (i.e., selenoproteins) varies from zero in higher plants to >30 in fish (Novoselov et al. 2002; Castellano et al. 2005; A.V. Lobanov and V.N. Gladyshev, unpubl.). Insertion of Sec requires the presence of a complex machinery that includes Sec tRNA, elongation factor EFsec, SECIS-binding protein 2 (SBP2), selenophosphate synthetase 2 (SPS2), phosphoseryl-tRNA kinase, SECp43, and Sec synthase (SecS), as well as a *cis*-acting stem–loop structure, the Sec insertion sequence (SECIS) element in the 3′-UTR (Hatfield et al. 2006).

Recently, we have shown that organisms with large selenoproteomes often associate with aquatic environments (Lobanov et al. 2007). As the Sec pathway is ancient, the first Sec-utilizing organisms likely lived in water. Mammals, insects, and certain other groups of organisms made successful and independent transitions from aquatic to terrestrial environments. In this process, some selenoproteomes were significantly reduced. Small

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terrestrial animals, such as fruit flies, have small selenoproteomes (Castellano et al. 2001; Martin-Romero et al. 2001), and *Caenorhabditis elegans* has only a single selenoprotein (Taskov et al. 2005). In higher plants and fungi, selenoproteins were completely lost.

An additional protein implicated in the pathway of Sec biosynthesis is selenophosphate synthetase 1 (SPS1) (Low et al. 1995). Previous research has shown that SPS1 has an important function in fruit flies and participates in oxidative stress defense (Morey et al. 2003a,b). Moreover, SPS1 knockout is lethal in flies, and a mutation in the SPS1 gene leads to larval lethality and increased apoptosis (Alsina et al. 1998). It is thought that selenophosphate synthetases, including eukaryotic SPS1, SPS2, and prokaryotic SelD, generate selenophosphate, an activated biological form of selenium. Many organisms utilizing Sec, such as mammals and fish, possess both SPS1 and SPS2, the latter itself a selenoprotein. It was reported (Kim et al. 1997) that replacement of Sec in mouse SPS2 with Cys reduced enzyme activity but did not completely inactivate the enzyme. SPS1 was also suggested to be responsible for Sec recycling (Tamura et al. 2004), and has been reported to interact with SecS in vitro and in vivo and coimmunoprecipitate with SecS and SecP43 from nuclear and cytosolic fractions (Small-Howard et al. 2006).

However, *Drosophila* SPS2 was found to synthesize selenophosphate from selenide in in vitro assays, whereas SPS1 did not support this reaction (Persson et al. 1997; Xu et al. 2006). Moreover, recent studies showed that knockdown of SPS1 in mammalian cells had no effect on selenoprotein biosynthesis, whereas knockdown of SPS2 severely impaired selenoprotein expression (Xu et al. 2007). In addition, mammalian SPS2 was active in synthesis of selenophosphate, whereas SPS1 was not active (Xu et al. 2006).

If SPS1 is involved exclusively in Sec biosynthesis, the mutation of this gene may have a phenotype similar to that resulting from disruption of the overall Sec pathway. However, while SPS1 is essential in fruit flies, disruption of the eEFsec gene had no effect on viability, and in fact, life spans of mutant and wild-type flies were similar (Hirosawa-Takamori et al. 2004). Thus, despite numerous studies, the role of SPS1 in selenoprotein biosynthesis remains unresolved.

In this work, we analyzed the selenoproteomes of aquatic and terrestrial arthropods and found that they were selectively reduced in insects. In the course of this study, we identified the first animals that do not have Sec-containing proteins and have also lost the Sec biosynthesis and insertion machinery. The presence of SPS1 in these organisms indicates that this protein is involved in a pathway unrelated to selenoprotein biosynthesis.

Results and Discussion

Reduced selenoproteomes in insects

Several computational approaches have been developed in recent years for the identification of selenoprotein genes (Kryukov et al. 1999; Lescure et al. 1999; Castellano et al. 2001). The genome of *D. melanogaster* was one of the first analyzed, and found to contain three selenoprotein genes (Castellano et al. 2001; Martin-Romero et al. 2001). Comparative analysis of *Drosophila pseudoobscura* and *D. melanogaster* genomes identified the same three selenoproteins (i.e., SPS2, BthD, and G-rich) (Lobanov et al. 2007). In the current work, we examined the selenoprotein content of other species of insects for which completely sequenced genomes are available as well as that of other arthropods.

Interestingly, aquatic arthropods had many more selenoproteins than their terrestrial counterparts even though none of them had their genomes fully sequenced (Fig. 1). Selenoproteins detected in aquatic arthropods were previously identified in vertebrates and green algae. However, in insects, many of these proteins were present in the form of Cys-containing homologs. As a result, all selenoproteomes of terrestrial arthropods, including *Anopheles gambiae*, *Apis mellifera*, *D. pseudoobscura*, *D. melanogaster*, *B. mori*, and

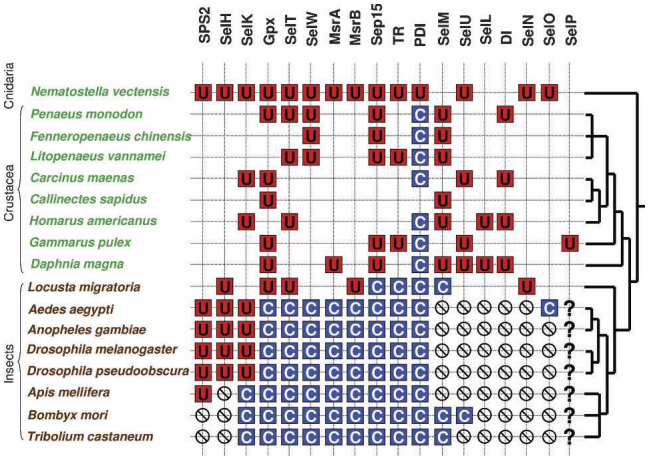

Figure 1. Occurrence of selenoproteins in insects and other arthropods. Eighteen selenoprotein families detected in any arthropod sequences or in a reference organism, cnidarian *Nematostella vectensis*, are shown at the *top* of the figure. Organism names shown in brown and green correspond to terrestrial and aquatic animals, respectively. Black "U" on red background shows the presence of Sec-containing proteins, and white "C" on blue background the presence of Cys-containing homologs, in the indicated organisms. Events of selenoprotein loss are shown by crossed black circles. Unmarked cells correspond to unfinished genomes, unclear evolutionary relationships among detected proteins, and possible expansion of selenoproteins in narrow groups of organisms. Question marks are used for selenoprotein P (SelP) sequences to indicate a currently unresolved evolutionary scenario.

Tribolium castaneum, were small and had 0–3 selenoproteins.

In addition to having Cys versions of selenoproteins, many insects lost several selenoproteins altogether, including SelH, SelU, and SelM. The widespread occurrence of these proteins in eukaryotes (Table 1), including arthropods, and their lack in all or a subset of insects, suggests loss of these proteins in insects rather than their gain in other organisms. Similarly, the widespread occurrence of other aquatic arthropod selenoproteins in eukaryotes and the presence of their Cys-containing homologs in insects suggest replacement of Sec with Cys in insect proteins (Fig. 1). Thus, there was a gradual reduction in the number of selenoproteins in insects. For example, *Locusta migratoria* has at least five selenoproteins, fruit flies and mosquitoes three, and the honeybee only one detected candidate selenoprotein.

Animals that lack selenoproteins

Interestingly, our selenoprotein searches revealed no selenoproteins in *B. mori* and *T. castaneum*. *T. castaneum*, the red flour beetle, is a model organism widely used in population ecology and genetics as well as in molecular and developmental genetics (Brown et al. 2002). *B. mori*, ''silkworm of the mulberry tree,'' is a popular model for insect genetics, second only to the fruit fly (Xia et al. 2004). The lack of selenoproteins in these organisms was surprising since all previously examined animals had selenoproteins. Since it was possible that our search strategy did not identify some selenoproteins, we analyzed genomic sequences for the presence of different components of Sec biosynthesis and incorporation machinery.

The use of tRNAscan-SE, a standard program for identification of tRNA genes, did not detect Sec tRNA in *B. mori* and *T. castaneum*, whereas a similar search found such structures in several other examined insects. We also carried out an alternative search that utilized BLASTN against insect tRNA sequences and an independent tRNA search program, Aragorn (Laslett and Canback 2004), but Sec tRNA again could not be found. We further analyzed for occurrence of SBP2, SECp43, PSTK, and SecS in arthropods (Table 2). These proteins could be detected in all tested insects with the exception of *B. mori* and *T. castaneum*. (In addition, the honeybee had some components of the Sec machinery but several components were not detected. Whether this organism is capable of synthesizing Sec-containing proteins is not clear.) The lack of components of Sec biosynthesis and insertion machinery in *B. mori* and *T. castaneum* is indicative of the loss of this machinery during evolution. Thus, results of the searches for these components are consistent with the lack of selenoproteins in these organisms. As a whole, these data provide evidence that *B. mori* and *T. castaneum* are the first known animals that do not synthesize Sec-containing proteins.

Interestingly, a recent study reported searches for selenoprotein genes in the *B. mori* genome, and claimed the identification of five selenoprotein genes containing in-frame UGA codons, including glutathione-S-transferase and four novel selenoproteins (Ping et al. 2006). However, examination of the structures reported in that study revealed that the SECIS-like structures identified in the 3′-UTRs of all five transcripts do not fit the canonical SECIS model, and could only be found with very relaxed settings of SECISearch. This mode can only be implemented for the identification of unusual SECIS elements and has a high false positive rate. In addition, all candidate *B. mori* selenoproteins have weak homology in the region flanking putative Sec residues, whereas in true selenoproteins this region tends to be highly conserved. In any event, the lack of any identifiable components of Sec biosynthesis and insertion machinery is a strong argument against the use of Sec in these predicted proteins.

SPS1 functions in a pathway unrelated to selenoprotein biosynthesis

Although *B. mori* and *T. castaneum* lacked both selenoprotein genes and Sec machinery, we detected SPS1 genes in these organisms (Table 2). The introduction of this paper contains an overview of the previous studies that examined the roles of selenophosphate synthetases in Sec biosynthesis. Critical analysis of these data indicates that the role of SPS2 in this pathway is well established, whereas the role of SPS1 is controversial. Our comparative genomics data clearly show that SPS1 is preserved even if no Sec-containing proteins are present in animals. Thus, this protein must function in a pathway unrelated to Sec biosynthesis. Such a Sec-independent pathway is essential in fruit flies (e.g., patufet mutation, a P-element insertion in the SPS1 gene that is lethal in homozygous flies at larval and pupal stages and also leads to a marked disruption in the size and morphology of the imaginal disks). It would be interesting to determine what the nature of this pathway is and whether selenium has a role in it. SPS1 may be involved in redox homeostasis, as previous data showed that it protects fruit flies from ROS damage (Morey et al. 2003b) and its antioxidant activity is dependent on p53 in mammalian cells (Chung et al. 2006).

Conclusions

Arthropods were found to possess selenoproteins that also could be found in other animals and unicellular eukaryotes. However, whereas many Sec-containing proteins

Table 1. *Selenoproteomes of representative eukaryotic organisms*

Organisms	Selenophosphate synthetase 2	Protein disulfide isomerase-like	Iodothyronine deiodinase (DI)	Sep15	Membrane selenoprotein	Glutathione peroxidase (GPx)	Thioredoxin reductase (TR)	Methionine-S-sulfoxide reductase (MsrA)	Methyltransferase	Peroxiredoxin (Prx)	Thioredoxin-fold protein	Selenoprotein H (SelH)	Selenoprotein I (SelI)	Selenoprotein K (SelK)
B. mori														
C. elegans							1							
C. reinhardtii					1	2	1	1				1		1
D. discoideum	1		1	1	1									1
D. melanogaster	1											1		1
D. pseudoobscura	1											1		1
H. sapiens	1		3	1		5	3					1	1	1
O. lucimarinus		3		1	1	5	1	1	1	3	1	1		1
O. tauri		3		1	1	5	1	1	1	1	1	1		1
T. pseudonana	1	2				2	1	1	1	2	1			
T. castaneum														

Organisms	Selenoprotein M (SelM)	Selenoprotein N (SelN)	Selenoprotein O (SelO)	Selenoprotein P (SelP)	Selenoprotein R (SelR, MsrB)	Selenoprotein S (SelS)	Selenoprotein T (SelT)	Selenoprotein U (SelU)	Selenoproteins W and V	Hypothetical protein 1	Hypothetical protein 2	Hypothetical protein 3	Total
B. mori													0
C. elegans													1
C. reinhardtii	1						1	1	2				12
D. discoideum													5
D. melanogaster													3
D. pseudoobscura													3
H. sapiens	1	1	1	1	1	1	1		2				25
O. lucimarinus	1		1			1	1	1	2	1	1	1	29
O. tauri	1		1			1	1	1	1	1	1	1	26
T. pseudonana	2						1	2					16
T. castaneum													0

Hypothetical proteins 1–3 refer to *Ostreococcus* selenoproteins described in Lobanov et al. (2007).

Table 2. *Occurrence of SPS1 and components of Sec insertion machinery in insects*[a]

Organism	SPS1	SPS2	Sec tRNA	SecS	PSTK	SBP2	SECp43	EFsec
Aedes aegypti	AAGE02015351	AAGE02011674		AAGE02006817	AAGE02003778	AAGE02021018	AAGE02022016	AAGE02006420
Anopheles gambiae	AAAB01008964	AAAB01008966		AAAB01008888	AAAB01008807	AAAB01008799		XP_316316
Apis mellifera	NW_001253018			XM_625120		XP_001122154		
Bombyx mori	AADK01042586							
Drosophila ananassae	AAPP01018789	AAPP01015727	AAPP01018708	APP01019594	AAPP01019757	AAPP01019357	AAPP01015985	AAPP01018740
Drosophila erecta	AAPQ01007059	AAPQ01007348	AAPQ01007068	AAPQ01006439	AAPQ01006016	AAPQ01006550	AAPQ01007326	AAPQ01007048
Drosophila grimshawi	AAPT01021568	AAPT01020944	AAPT01020758	AAPT01020190	AAPT01019159	AAPT01020544		AAPT01021484
Drosophila melanogaster	AABU01002766	NP_477478	NT_033778.3	NP_996155	AABU01002771	NT_037436	NT_033779	AABU01002765
Drosophila mojavensis	AAPU01010156	AAPU01010712	AAPU01010253	AAPU01011109	AAPU01007942	AAPU01011517	AAPU01010675	AAPU01010147
Drosophila persimilis	AAIZ01008061	AAIZ01000317	AAIZ01002024	AAIZ01000577	AAIZ01010094	AAIZ01006749	AAIZ01003312	AAIZ01008678
Drosophila pseudoobscura	AADE01000386	AADE01000230	AADE01000308	AAFS01000039	AADE01001242	AADE01000255	AADE01000244	EAL26649
Drosophila sechellia	AAKO01000635	AAKO01001042	AAKO01000568	AAKO01001566	AAKO01001822	AAKO01000013	AAKO01001425	AAKO01001996
Drosophila simulans	AASW01034011	AASU01047131	AAST01002032	AASS01003771	AAST01022456	AASW01051719	AASW01003010	AAST01028952
Drosophila virilis		AANI01016111	AANI01014812	AANI01014650	AANI01016895	AANI01017351	AANI01016283	AANI01014934
Drosophila yakuba	AAEU02000421	AAEU02000111	AAEU02000426	AAEU02000051	AAEU02001551	AAEU02000132	AAEU02000372	AAEU02000410
Tribolium castaneum	NW_001092860							

[a] Accession numbers are shown if sequences were identified in organisms. Organisms containing SPS1, but lacking Sec insertion machinery, are shown in bold.

occur in aquatic arthropods, insects, which are terrestrial arthropods, have few such proteins. We show that there was a selective selenoproteome reduction in insects through the loss of some selenoprotein genes and conversion of other Sec-containing proteins into Cys-containing homologs. Our analysis revealed the first animals completely lacking both Sec biosynthesis and selenoprotein genes (e.g., the silkworm, *B. mori*, and the red flour beetle, *T. castaneum*). These animals, however, encode the *sps1* gene. Thus, SPS1 functions in a pathway unrelated to Sec biosynthesis. Our finding addresses the controversy regarding the function of SPS1 and suggests that future studies might search for a new pathway that utilizes this protein. Since SPS1 evolved from an ancestral SPS/SelD, a protein involved in the Sec pathway, an attractive possibility is that SPS1 may define a new pathway of selenium utilization in animals, including mammals.

Materials and Methods

EST, whole genome shotgun (WGS), and nonredundant (NR) data sets were obtained from NCBI. BLAST programs, also from NCBI, were used for homology searches. Human, fish, *Drosophila*, and Plasmodium sequences corresponding to known components of the Sec biosynthesis and insertion machinery were used as query sequences. *B. mori* sequences were obtained from the Silkworm Genome Database (http://www.ab.a.u-tokyo.ac.jp/genome/) and *T. castaneum* from Baylor College of Medicine (http://www.hgsc.bcm.tmc.edu/projects/tribolium/).

Analyses of selenoproteomes were carried out as described elsewhere (Kryukov et al. 2003). First, homologous sequences with UGA corresponding to Sec in known selenoproteins were identified using TBLASTN, and their 3′-UTRs were examined for the presence of SECIS elements. In addition, sequences satisfying primary sequence consensus were identified using SECISearch (Kryukov et al. 2003). Then, secondary structure criteria and additional filters were applied. SECIS candidates were further analyzed with BLASTN to identify structures conserved between different species, and TBLASTX was used to analyze their upstream regions for selenoprotein ORFs.

Aragorn (Laslett and Canback 2004), tRNAscan-SE (Schattner et al. 2005) and BLASTN programs were used to identify Sec tRNAs in analyzed genomes. Identified selenoproteins were placed in the phylogenetic scheme using taxonomy data and the Tree of Life web project (www.tolweb.org).

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