Journal of Insect Physiology 71 (2014) 46–51

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Selenophosphate synthetase in the male accessory glands of an insect without selenoproteins

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ARTICLE INFO

Article history:
Received 29 June 2014
Received in revised form 28 September 2014
Accepted 30 September 2014
Available online 11 October 2014

Keywords:
Selenoprotein
Selenophosphate synthetase 1
Male accessory gland proteins
Hymenoptera

ABSTRACT

Selenoproteins (containing the 21st proteinogenic amino acid selenocysteine) play important roles throughout all domains of life. Surprisingly, a number of taxa have small selenoproteomes, and Hymenopteran insects appear to have fully lost selenoproteins. Nevertheless, their genomes contain genes for several proteins of the selenocysteine insertion machinery, including selenophosphate synthetase 1 (SELD/SPS1). At present, it is unknown whether this enzyme has a selenoprotein-independent function, and whether the gene is actually translated into a protein in Hymenoptera. Here, we report that SELD/SPS1 is present as a protein in the accessory glands of males of the ant *Cardiocondyla obscurior*. It appears to be more abundant in the glands of winged disperser males than in those of wingless, local fighter males. Mating increases the lifespan and fecundity of queens in *C. obscurior*, and mating with winged males has a stronger effect on queen fitness than mating with a wingless male. SELD/SPS 1 has been suggested to play an important role in oxidative stress defense, and might therefore be involved in the life-prolonging effect of mating.

1. Introduction

Selenoproteins occur in almost all life forms, from Archaea and Bacteria to animals including humans. They contain the 21st proteinogenic amino acid selenocysteine in their reactive center and mainly function as redox-enzymes (Hamilton and Tappel, 1963; Stadtman, 1996). Selenocysteine is incorporated into proteins during translation, but in contrast to most other amino acids is not encoded by its own codon. Instead, an in-frame opal stop codon (UGA) is recoded, and a selenocysteine insertion element (SECIS-element) located at a specific distance from this codon activates several cofactors (e.g. SELA-SELD in Bacteria, (Böck et al., 1991a,b; Ehrenreich et al., 1992; Forchhammer et al., 1989); for Eukaryotes see (Turanov et al., 2011; Xu et al., 2006)) that act together as the selenocysteine insertion machinery. As selenium is more reactive than sulfur, proteins with selenocysteine residues show a greater reduction potential in the defense against reactive oxygen species (ROS) than homologous proteins containing cysteine residues (Battin and Brumaghim, 2009; Gladyshev, 2006).

With the increasing availability of insect genomes, it has become obvious that insects have strongly reduced selenoproteomes (Lobanov et al., 2007), probably because their ROS-defense works completely different from that of vertebrates (Kanzok et al., 2001) but see (Morey et al., 2003a,b; Pedersen et al., 2008). While some fruit fly species still have three functional selenoproteins (Adams et al., 2000; Castellano et al., 2001; Martin-Romero et al., 2001), there is strong evidence that Hymenoptera (ants, bees, wasps etc.) have lost them completely (Chapple and Guigó, 2008). However, several Hymenoptera still possess different, yet incomplete sets of genes of the selenoprotein biosynthesis machinery. Common to all presently sequenced genomes is the conserved gene selenophosphate synthetase 1 (also referred to as selenide, water dikinase, *sps1*, *selD*, (Persson et al., 1997), *ptuf*, (Alsina et al., 1998; Serras et al., 2001)), which encodes the enzyme that from selenide and ATP synthesizes selenophosphate, the selenium donor for the synthesis of selenocysteine (Chapple and Guigó, 2008). Aside from selenocysteine biosynthetic processes (Alsina et al., 1999; Tamura et al., 2004) the enzyme has been reported to be – in conjunction with selenoproteins – involved in various physiological processes like cell proliferation, imaginal disc development (Alsina et al., 1998, 1999; Serras et al., 2001), glutamine metabolism, mitochondrion organization (Shim et al., 2009; Lee et al., 2011), negative regulation of ROS homeostasis (Alsina et al., 1999; Morey et al., 2003a,b), stress responses (Morey et al., 2003a,b; Pedersen et al., 2008), and neurogenesis (Neumüller et al., 2011). Recent studies suggest an additional role in selenoprotein-biosynthesis (Alsina et al., 1999;

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http://dx.doi.org/10.1016/j.jinsphys.2014.09.012
0022-1910/

Tamura et al., 2004) and an as yet unknown, selenoprotein-unrelated function (Han et al., 2012; Lobanov et al., 2008; Xu et al., 2006, 2007).

Consequently, the gene, its evolutionary history and its particular function have become the subject of increasing attention. Despite of this growing interest in *selD/sps1* it has remained unclear, whether it is translated into a protein in those insects that do not have selenoproteins, and, if so, in which tissue and with which function. Even in *Drosophila willistoni*, the first animal reported to completely lack selenoproteins (Chapple and Guigó, 2008; Clark et al., 2007), it is not yet clear, whether genes residing in its genome for selenoprotein biosynthesis are translated at all, or which alternative function they might have.

Here we report on the occurrence of SELD/SPS1 in the male accessory glands (MAG) of the ant *Cardiocondyla obscurior*. In solitary insects, peptides and proteins transferred in the seminal fluid of males may harm the female and shorten its life expectancy. In contrast, *C. obscurior* queens that mated with normal or with sterilized males lived significantly longer than virgin queens, suggesting that compounds transferred in the seminal fluid positively affect their physiology (Schrempf et al., 2005). *C. obscurior* is characterized by a peculiar diphenism with winged, gracile males, which are produced when environmental conditions change and disperse, and wingless (so called ergatoid, i.e. "worker like") fighter males, which remain in their natal nests throughout their lives, attempt to kill all rival males with their saber-shaped mandibles and monopolize mating with the young queens (Heinze and Hölldobler, 1993). The observation that mating with a winged male increases queen lifespan even more than mating with a wingless male (Schrempf and Heinze, 2008) motivated a detailed comparison of the protein compositions of MAGs of the two types of males.

2. Materials and methods

2.1. Ant sampling and housing

Colonies of *C. obscurior* (Wheeler, 1929) were collected from an experimental coconut plantation in Una, Bahia, Brazil in July 2009. Ants were then housed in artificial nests and fed twice a week with chopped cockroaches (*Nauphoeta cinerea*), fruit flies (*Drosophila melanogaster*), and a drop of honey. To obtain winged and wingless males, colonies were transferred from a 12 h/12 h day/night – cycle at 23–27 °C into an incubator with permanent light and a constant temperature of 22 °C. Four to six weeks later the first winged male pupae could be observed and colonies started to produce a cohort of winged males together with wingless males.

2.2. Sample preparation and performance of the first dimension: Isoelectric focusing (IEF)

Winged males were collected twice a week and put together with a few workers in a separate nest, where they lived until they were dissected 7–10 days later to ensure that all males had mature accessory glands. Wingless males of *C. obscurior* ants engage in lethal fighting with wingless rivals and we therefore collected them with a minimum time interval of 7 days.

Males were dissected on a cooled object slide in a drop of Beadle-solution (128.3 mM NaCl, 4.7 mM KCl, and 2.3 mM $CaCl_2$ (Darrouzet et al., 2002)) and nine pairs of MAGs were collected in 20 μL DeStreak Rehydration Solution (GE Healthcare) with 2% IPG (pH = 3–11 NL, GE Healthcare) from either wingless or winged males. Samples were stored at −75 °C until usage. For each type of male, a total of ten gels, each loaded with the protein extract of nine pairs of MAGs, were run. The gel prepared for downstream nano-LC–MS/MS-analysis of excised protein spots contained 30 pairs of MAGs of wingless males in 40 μL DeStreak Rehydration Solution.

Equipment and solutions for the IEF were prepared following the manufacturer's protocol (Hoefer). Directly before starting the first dimension, the completely thawed samples were alternately sonicated and put on ice for 15 s each. This procedure was repeated four times, before adding 40 μL of rehydration solution (8 M Urea, 1% CHAPS, 13 mM DTT, 0.5% (v/v) Servalyt pH = 3–10 (Serva)). After thoroughly mixing, the samples were centrifuged at 16,100*g* for 12 min to avoid contamination with unsolved tissue. Only the supernatant was applied onto the gels-strips (11 cm IPG-strips (Serva) with a linear pH-gradient from 3 to 10). Isoelectric focusing was performed in an IEF100 (Hoefer) apparatus for a total of 15,000 Vh using the cup-loading method.

2.3. Preparation and performance of the second dimension: SDS–PAGE

For the reduction of disulfide bonds between cysteine residues the IPG-strips were incubated for 12 min in 6 mL of equilibration-solution (6 M urea, 75 mM Tris–HCl, pH = 8.8, 29.3% (v/v) glycerol, 2% (w/v) SDS; bromophenol blue) containing 1% (w/v) DL dithiothreitol (DTT). To avoid the renewed formation of disulfide bonds, the thiol groups of the cysteine residues were alkylated in a second step by addition of equilibration solution containing 2.5% (w/v) iodoacetamide (IAA) instead of DTT.

The second dimension was conducted on a SE900 (Hoefer). Again equipment and solutions were prepared following the manufacturer's manual. An ESP 601 (Amersham Pharmacia Biotech) power supply was employed as power source. Instead of the suggested 480 mA (80 mA/gel), a maximum of 400 mA was applied for a run with six gel-plates (settings: 400 mA, 600 V, 100 W). Proteins were separated on the basis of their size in homogeneous 2500 × 2100 × 1 mm 12.5% SDS–polyacrylamide gels cast by us. The Tris–glycine buffer as described by Laemmli was used (Laemmli, 1970). First and second dimension runs were always performed on the same day. After additional manual matching to the reference gel of each phene, statistical analysis was carried out using the Progenesis SameSpots software (Nonlinear Dynamics Limited), which itself does an automatic matching to a previously selected reference gel.

2.4. Silver staining

For silver staining SDS–PAGE gels were removed from the glass plates and placed separately overnight into 200 mL of fixation-solution for silverstaining, containing 10% (v/v) acetic acid. For maximum staining sensitivity in the nanogram range, gels were treated after a water wash with a 5% (v/v) glutaraldehyde solution for 30 min prior to silver staining (Blum et al., 1987). Glutardialdehyde fixation of proteins is not suited for excision and trypsination of protein spots prior to subsequent mass-spectrometric analysis. For that purpose, silver staining without glutardialdehyde was used.

2.5. Nano-LC–MS/MS-analysis

Protein spots of interest were excised and subjected to tryptic digestion and subsequent nano-LC–MS/MS as published previously (Thomas et al., 2013). The MS/MS-spectra were searched against the Uniprot database using the Mascot Daemon and the Mascot algorithm (version 2.2; Matrix Science Ltd., London, UK) using trypsin as protease with max. one missed cleavage site, carbamidomethylation of cysteines as constant, methionine oxidation as variable modification and 0.2 Da-tolerance for MS- and MS/MS-signals.