Journal of Insect Physiology 59 (2013) 351–366

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

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

Insights into the insect salivary gland proteome: Diet-associated changes in caterpillar labial salivary proteins

Khashayar Afshar [a], Fitsum Fikru Dube [a], Hamed S. Najafabadi [b], Eric Bonneil [c], Pierre Thibault [c], Reza Salavati [b], Jacqueline C. Bede [a,*]

[a] Department of Plant Science, McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, Qc, Canada H9X 3V9
[b] Institute of Parasitology, McGill University, 21,111 Lakeshore, Ste-Anne-de-Bellevue, Qc, Canada H9X 3V9
[c] Institute for Research in Immunology and Cancer, Université de Montreal, Montréal, Qc, Canada H3T 1J4

ARTICLE INFO

Article history:
Received 21 August 2012
Received in revised form 5 January 2013
Accepted 7 January 2013
Available online 23 January 2013

Keywords:
Beet armyworm
Labial salivary gland
NanoLC/ESI/MS/MS
Nutritional quality
Proteomics
Spodoptera exigua

ABSTRACT

The primary functio of salivary glands is fluid and protein secretion during feeding. Compared to mammalian systems, little is known about salivary protein secretion processes and the effect of diet on the salivary proteome in insect models. Therefore, the effect of diet nutritional quality on caterpillar labial salivary gland proteins was investigated using an unbiased global proteomic approach by nanoLC/ESI/tandem MS. Caterpillars of the beet armyworm, *Spodoptera exigua* Hübner, were fed one of three diets: an artificial diet containing their self-selected protein to carbohydrate (p:c) ratio (22p:20c), an artificial diet containing a higher nutritional content but the same p:c ratio (33p:30c) or the plant *Medicago truncatula* Gaertn. As expected, most identified proteins were associated with secretory processes and not influenced by diet. However, some diet-specific differences were observed. Nutrient stress-associated proteins, such as peptidyl-propyl *cis–trans* isomerase and glucose-regulated protein94/endoplasmin, and glyceraldehyde 3-phosphate dehydrogenase were identified in the labial salivary glands of caterpillars fed nutritionally poor diets, suggesting a link between nutritional status and vesicular exocytosis. Heat shock proteins and proteins involved in endoplasmic reticulum-associated protein degradation were also abundant in the labial salivary glands of these caterpillars. In comparison, proteins associated with development, such as arylphorin, were found in labial salivary glands of caterpillars fed 33p:30c. These results suggest that caterpillars fed balanced or nutritionally-poor diets have accelerated secretion pathways compared to those fed a protein-rich diet.

1. Introduction

Recognition that insect oral secretions (saliva and/or regurgitant) modify host plant or animal responses has lead to proteomic investigation of the salivary gland (sialome) or associated secretions (secretome). In blood-feeding insects, often vectors of disease-causing parasites, identification of salivary proteins may lead to targets for the control of transmission and/or disease (Ribeiro and Francischetti, 2003; Valenzuela et al., 2003; Ribeiro et al., 2004; Kalume et al., 2005; Arcà et al., 2007). In phloem-feeding insects, salivary recognition factors responsible for the initiation of host plant defenses have been identified (Harmel et al., 2008; Carolan et al., 2009; Cooper et al., 2010). However, given the importance of caterpillar saliva as a potential source of enzymes for the digestion and detoxification of noxious compounds as well as effectors which suppress the induction of plant defenses, few studies have focused on the caterpillar labial salivary gland and proteins involved in secretory processes (Mathews et al., 1997;

Abbreviations: BiP, binding immunoglobulin protein/glucose regulated protein GRP78; COPI, coat protein complex I; COPII, coat protein complex II; EE, early endosome; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum-associated degradation; ERGIC, endoplasmic reticulum Golgi intermediate compartment; GA3PDH, glyceraldehyde 3-phosphate dehydrogenase; GOX, glucose oxidase; GRP94, glucose regulated protein 94; HSP, heat shock protein; IDGF, imaginal disc growth factor; nanoLC/ESI/MS/MS, nanoliquid chromatography/electrospray ionization/mass spectroscopy/mass spectroscopy; LE, late endosome; NCBI, National Center for Biotechnology Information; P26S4, proteasome 26 subunit 4; p97/Npl4/Ufd1 complex, p97/nuclear protein localization 4/ubiquitin fusion degradation 1 complex; PDI, protein disulphide isomerase; PPI, peptidyl-propyl *cis–trans* isomerase; RPN, regulatory particle non-ATPase; TBP1, ATP-dependent TAT binding protein-1; TGN, *trans*–Golgi network; RACK1, receptor for activated protein C kinase; V-ATPase, vacuolar-ATPase.

* Corresponding author. Tel.: +1 514 398 7860.
E-mail addresses: Khashayar.afshar@mail.mcgill.ca (K. Afshar), Fitsum.dube@mail.mcgill.ca (F.F. Dube), Hamed.shaterinajafabadi@mail.mcgill.ca (H.S. Najafabadi), Eric.bonneil@umontreal.ca (E. Bonneil), Pierre.thibault@umontreal.ca (P. Thibault), Reza.salavati@mcgill.ca (R. Salavati), Jacqueline.bede@mcgill.ca (J.C. Bede).

0022-1910/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jinsphys.2013.01.002

Eichenseer et al., 1999; Musser et al., 2002a; Weech et al., 2008; Zhou et al., 2008; de la Paz Celorio-Mancera et al., 2011, 2012).

Lepidopteran larvae possess two distinct salivary organs; a pair of labial salivary glands and a pair of mandibular salivary glands (House and Ginsborg, 1985). The watery secretions of the mandibular glands contain proteins, lipids, sterols and triglycerides but little is known about its full composition (Felton, 2008). Recent proteomic analysis of *Vanessa gonerilla* caterpillar mandibular glands identified key proteins, such as lysozyme, α-amylase, a putative chemosensory protein and sericotropin, associated with the mandibular salivary glands (de la Paz Celorio-Mancera et al., 2012). Even though the labial salivary glands of some caterpillar species, such as the silkworm, *Bombyx mori*, are specialized for silk production, these glands in many other caterpillar species are responsible for proteinaceous salivary secretions (Eichenseer et al., 2010). Structurally, caterpillar labial salivary glands are a pair of long, unicellular, tubular structures that fuse to form a common duct in the head region (House and Ginsborg, 1985; Parthasarathy and Gopinathan, 2005; Daimon et al., 2008). After secretory cells release enzymes, ions and water into the salivary gland lumen, ions are retaken up by the resorptive cells, and then the saliva is finally released from the labial salivary duct through a specialized organ, the spinneret (House and Ginsborg, 1985; Ali, 1997; Musser et al., 2002a). In caterpillar species whose labial glands mainly produce protein-rich saliva, important enzymes, including lysozyme, ascorbate peroxidase and glucose oxidase (GOX), in these secretions have been characterized (Mathews et al., 1997; Eichenseer et al., 1999; Liu et al., 2004; de la Paz Celorio-Mancera et al., 2011, 2012). However, in caterpillars, little is known about protein secretion processes in these glands.

Saliva formation and salivation are highly regulated processes. In human parotid cells, a number of pathways are implicated in proteinacous salivary secretion (Gorr et al., 2005). The classical secretory pathway is predominantly responsible for protein-rich secretions resulting from the exocytosis of large dense-core secretory vesicles, which may be constitutively secreted (minor) or stimulated in response to muscurinic-cholinergic and adrenergic signals. Pathways responsible for minor salivary secretions include a constitutive pathway where the secretory vesicles are derived from the *trans*–Golgi network and a regulated pathway where further maturation of the secretory granules occurs. In the main classical secretory pathway, proteins destined for salivary secretions through the *trans*–Golgi network and/or dense core secretory vesicles are synthesized by ribosomes and translocated into the ER lumen through the Sec translocon complex co-translationally or, less commonly, post-translationally (Kim et al., 2006; Gasser et al., 2008; Brunner et al., 2009). In the ER lumen, nascent polypeptide chains follow one of two major pathways (Braakman and Bulleid, 2011). One route involves the binding of the growing polypeptide chain to the Binding immunoglobulin Protein/Glucose-regulated Protein 78 kD/Heat Shock Protein 70 (BiP/GrP78/HSP70) followed by formation of disulfide bonds by a protein disulfide isomerase (PDI). For glycoproteins, *N*-linked monoglycans are recognized by the ERp57/calreticulin complex or canexin (Ellgaard and Helenius, 2003; Frickel et al., 2004). Only upon proper folding are proteins transported to the *trans*–Golgi network. Misfolded proteins remain bound to BiP which leads to retrograde translocation into the cytosol and proteasome-mediated protein degradation through the ER-associated degradation (ERAD) pathway (Nishikawa et al., 2001; Ryoo and Steller, 2007; Bagola et al., 2011). From the Golgi, a number of cellular pathways lead to secretory granular biogenesis and the extracellular release of proteinaceous material (Burgoyne and Morgan, 2003; Nashida et al., 2004; Gorr et al., 2005). The exact mechanism for the formation of dense-core granules from the *trans*–Golgi network is not fully understood but proposed models include the vesicular transport or the cisternal maturation or the cisternal progenitor models (Gorr et al., 2005; Pfeffer, 2010).

In response to adrenergic or muscarinic-cholinergic signals, secretory granules in salivary glands are guided to the plasma membrane on microtubule rails driven by kinesin protein motors (Hirokawa et al., 1998; Nashida et al., 2004). At the plasma membrane, granules transport then switches to an actin filament (microfilaments)/myosin system (Valentijn et al., 1999). Release of granule contents into the gland lumen may involve a rapid fusion and reclosure of the fusion pore (kiss-and-run model) or full fusion and emptying of the granule with the plasma membrane followed by retrieval involving a clathrin/dyamin-associated mechanism (Harata et al., 2006).

In diverse insect species, diet affects either salivary protein levels or activity or secretion. In female mosquitoes, blood-feeding rapidly results in dynamic changes to the salivary gland transcriptome (Das et al., 2010). Salivary secretions of the Russian wheat aphid, *Diuraphis noxia*, were also affected by diet (Cooper et al., 2010). In Noctuid caterpillars, a number of salivary enzymes have shown diet-specific gene expression and activity (Liu et al., 2004; Afshar et al., 2010). Mid-5th instar corn earworm, *Helicoverpa zea*, salivary glands have higher expression of the gene encoding lysozyme when caterpillars feed on cotton or tomato compared to tobacco plants (Liu et al., 2004). The effect of diet on caterpillar labial salivary GOX activity is of particular interest since GOX potentially negatively affects the plant's ability to mount an appropriate defense response (Eichenseer et al., 1999, 2010; Musser et al., 2002a; Weech et al., 2008). GOX catalyses the oxidation of glucose and the activity of this enzyme have been found to increase when caterpillars were fed carbohydrate-based diets, lending credibility to the idea that this enzyme may be a pre-ingestive mechanism to balance dietary sugar intake as excess carbohydrates have a negative impact on insect mortality (Felton, 1996; Warbrick-Smith et al., 2006; Babic et al., 2008). Caterpillars often exhibit a self-selective feeding behavior to balance their diet, in particular, the intake of protein to digestible carbohydrate (p:c) ratio, which leads to optimal performance (growth, development, fecundity) of the insect (Waldbauer and Friedman, 1991; Lee et al., 2002; Merkx-Jacques et al., 2008); the self-selected ratio of *Spodoptera exigua* Hübner caterpillars is 22p:20c (Merkx-Jacques et al., 2008). By altering the ratio and levels of p:c, Afshar et al. (2010) showed that labial salivary gland SeGOX gene expression reflects glucose levels, but enzyme activity is also influenced by the nutritional protein content such that enzyme activity was highest when caterpillars were fed a carbohydrate- and protein-rich diet (Afshar et al., 2010). This suggests that there is both transcriptional and post-translational regulation of GOX. However, an alternative explanation is that on the high carbohydrate diet, GOX protein levels and salivary secretions increase but on a high protein diet, a feedback loop inhibits secretory granule exocytosis leading to the accumulation of labial salivary digestive enzymes.

In many insect models, diet and, in particular, nutritional quality, alters salivary enzyme gene expression, activity and secretion (Liu et al., 2004; Merkx-Jacques and Bede, 2005; Hu et al., 2008; Afshar et al., 2010; Das et al., 2010). This study examined the role of diet on caterpillar salivary secretion machinery by using a global proteomic approach. Three diets were chosen: the plant *Medicago truncatula* L. and two artificial diets that varied in their protein and carbohydrate levels, while maintaining the same protein to carbohydrate (p:c) ratio. *M. truncatula* was chosen as it is eaten by caterpillars of the beet armyworm, *S. exigua*, a generalist Noctuid pest. The self-selected p:c ratio by these caterpillars is 22p:20c (Merkx-Jacques et al., 2008). Therefore, the two artificial diets (22p:20c and 33p:30c) represent this ratio; previous studies have shown SeGOX gene expression and enzyme activity is higher on 33p:30c (Afshar et al., 2010). *S. exigua* caterpillars were reared