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Short communication

Profiling the proteome complement of the secretion from hypopharyngeal gland of Africanized nurse-honeybees (*Apis mellifera* L.)

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Abstract

The protein complement of the secretion from hypopharyngeal gland of nurse-bees (*Apis mellifera* L.) was partially identified by using a combination of 2D-PAGE, peptide sequencing by MALDI-PSD/MS and a protein engine identification tool applied to the honeybee genome. The proteins identified were compared to those proteins already identified in the proteome complement of the royal jelly of the honey bees. The 2-D gel electrophoresis demonstrated this protein complement is constituted of 61 different polypepides, from which 34 were identified as follows: 27 proteins belonged to MRJPs family, 5 proteins were related to the metabolism of carbohydrates and to the oxido-reduction metabolism of energetic substrates, 1 protein was related to the accumulation of iron in honeybee bodies and 1 protein may be a regulator of MRJP-1 oligomerization. The proteins directly involved with the carbohydrates and energetic metabolisms were: alpha glucosidase, glucose oxidase and alpha amylase, whose are members of the same family of enzymes, catalyzing the hydrolysis of the glucosidic linkages of starch; alcohol dehydrogenase and aldehyde dehydrogenase, whose are constituents of the energetic metabolism. The results of the present manuscript support the hypothesis that the most of these proteins are produced in the hypoharyngeal gland of nurse-bees and secreted into the RJ.

Keywords: Africanized *Apis mellifera*; Royal jelly; Peptide mass fingerprint; Proteome; MALDI-TOF

1. Introduction

The honeybee (*Apis mellifera* L.) is a social insect, living in colonies constituted of different castes: a queen, workers and drones (Lercker et al., 1982; Palma, 1992). The age-dependent role is one of the most notable features of the workers in these colonies (Ohashi et al., 1999). Young workers, known as nurse-bees take care of the brood, by synthesizing and secreting many components of the royal jelly (RJ), while the older workers usually forage for nectar, converting it into honey (Robinson, 1987). This process is biologically regulated and known as age polyethism, which is paralleled by physiological changes in certain organs of the worker honeybees (Ohashi et al., 1997).

The RJ is believed to be synthesized both by the mandibular and hypopharyngeal glands of nurse-honeybees (Knecht and Kaatz, 1990; Lensky and Rakover, 1983). The hypoharyngeal gland is well developed in the nurse-bees, but shrinks in the older workers, to adapt

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the insect to forage and honey production (Ohashi et al., 1999). Thus, it may be assumed that the gland synthesizes different proteins according to age polyethism (Kubo et al., 1996).

Taking into account that the secretion of this gland constitutes an important part of the RJ composition, it is necessary to identify the biochemical contributions of the hypopharyngeal gland to the composition of the RJ, in order to get a better understanding the role of this gland for the brood care.

The RJ contains proteins, free amino acids, fatty acids, sugars, vitamins, some minerals (Palma, 1992) and constitutes the principal food of the queen honeybees; also it is part of the initial diet of honeybee larvae (Moritz and Southwick, 1992). The protein content represent up to 12% (m/m) of freshly harvested RJ and the major components seem to constitute members of the most known family of proteins of this secretion, the major royal jelly proteins (MRJP) (Knecht and Kaatz, 1990; Lensky and Rakover, 1983); this family of proteins represent about 82% of the total protein content of RJ (Schimitzová et al., 1998).

The complexity of the protein complement composition of the RJ and the absence of fundamental sequential data about the most of genes involved with the expression of these proteins, make their biochemical and physiological characterizations difficult, which in turn makes the understanding of their functions not fully known. In addition to this, there has been few substantial biochemical analysis of the proteins synthesized by the hypopharyngeal gland.

Four major proteins (50, 56, 57 and 64 kDa) were demonstrated to be selectively synthesized by the hypopharyngeal gland and secreted as RJ proteins (Hanes and Simúth, 1992; Kubo et al., 1996). These results constitute strong evidences that the some proteins found in RJ are in fact products of the hypopharyngeal gland secretion.

Recently, the basic composition of the RJ was analyzed by a proteomic approach, which identified the presence of several different forms of MRJP-1 to MRJP-5 and glucose oxidase, presenting heterogeneities in terms of molecular weights and isoelectric points (Sato et al., 2004). This study offered a wide view of the composition of the proteome complement of the RJ, opening the possibility to develop a similar investigation with the hypopharyngeal gland, in order to compare both compositions considering a large number of proteins. Thus, the aim of the present study was to obtain a proteome profile from the secretion of hypopharyngeal gland of nurse-bees (*A. mellifera*) by using the honeybee (HB) genome databank as reference for proteins identification and to compare the proteins identified with those already described in RJ, in order to get a better understanding about the contribution of this gland to the composition of the RJ.

2. Material and methods

2.1. Insects and the collection of the secretion from hypopharyngeal-gland

Africanized honeybees (*A. mellifera* L.) kept in the apiary of the Institute of Biosciences of São Paulo State University, at Rio Claro, southeast Brazil, were used. Newly emerged workers were marked (day-0) on their thorax using paint and introduced into a normal queenright colony; marked nurse bees were collected 7 days later when they were feeding brood. In order to extract proteins of the hypopharyngeal gland secretion the bees were anesthetized by using carbonic gas and the hypopharyngeal glands were dissected under a binocular microscope. The glands (500 glands/ml) were washed in buffered saline solution (10 mM Tris-HCl pH 7.4, containing 13 mM NaCl, 5 mM KCl and 1 mM $CaCl_2$), containing a mixture of protease inhibitors: 1 mM phenylmethylsulfonyl fluoride, 0.1 μg/ml pepstatin and 100 μg/ml leupeptin as described elsewhere (Kubo et al., 1996). The washing extracts were centrifuged at $2000 \times g$ during 15 min at 4 °C.

The supernatant was collected and the debris discarded; the supernatant was then filtered through a Millex membrane (0.45 μm pore diameter) at 4 °C, lyophilized and dissolved in 1 ml urea/thiourea buffer (2 M thiourea, 7 M urea, 4 w/v DTT, 2% v/v carrier ampholytes pH 3–10). Protein content was determined by using the modified method of Bradford (Sedmak and Groosber, 1977).

2.2. D-polyacrylamide gel electrophoresis (PAGE)

2-D PAGE was performed by using 13 cm gels and IPG strips with immobilized pH gradients (IPG_Dalt); for the first dimension (IPG-IEF) IPG gels were cast with pH gradients of 3–10 (Görg et al., 1999, 2000). Samples (25 μl) were applied by cup-loading close to the anode and the focusings were performed by using the following voltage gradient: 500 V/1 h, 1000 V/1 h and 8000 V/2 h permitting the accumulation of 16 000 V h. The runnings were performed at 20 °C, under a current of 0.05 mA with a maximal power of 5.0 W. After equilibration of the IPG strips with SDS buffer were applied to vertical SDS-PAGE gels [12.5% (w/v) polyacrylamide and 0.8%(w/v) Bis(*N*,*N*′-methylenebisacrylamide)]. The second dimension runnings were performed according to the following program: 5 W/gel during 30 min and 17 W/gel during 5 h, at 10 °C. After the electrophoretic separation the gels were stained with Coomassie Brilliant Blue (CBB) and stored at room temperature (21 °C).