The intramandibular gland of leaf-cutting ants
(Atta sexdens rubropilosa Forel 1908)

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Abstract

The eusociality developed in Hymenoptera and Isoptera is driven by an efficient interaction between exocrine glands and jointed appendages, both in close interaction with the environment. In this context, the mandible of ants plays an important role, since, in addition to being the main jointed appendage, it possess glandular functions. As an example we might name the two glands associated with the mandible: the mandibular and the intramandibular glands. The intramandibular gland is found inside the mandible and consists of a hypertrophied secretory epithelium and secretory cells in the mandible’s lumen. The secretion of the secretory epithelium is liberated through intracuticular ducts that open at the base of hairs at the mandible’s surface. The secretion of the intramandibular gland (epithelium and secretory cells) reacted positively to tests for the detection of polysaccharides and proteins, thus suggesting that it consists of glycoproteins. The ultrastructure of the secretory epithelium presents variations related to the developmental stage of the individual, showing a large number of ribosomes and microvilli close to the cuticle in young individuals, while in the older specimens it was possible to note the formation of an intracellular reservoir. These variations of secretory epithelium, as also the interaction between the cellular groups inside the mandible, are important information about this gland in leaf-cutting ants.

Keywords: Hymenoptera; Social insects; Exocrine glands; Ultrastructure; Histology; Histochimistry

1. Introduction

The eusociality in certain groups of insects (orders Isoptera and Hymenoptera) is characterized by the interdependence among the members of the colony (Hölldobler and Wilson, 1990). This characteristic is caused by the interaction of exocrine glands and efficient jointed appendages with the environment and with other individuals of the colony. The leaf-cutting ant (Atta sexdens rubropilosa) deserves special attention in this context since it possesses one of the most complete communication systems of the Formicidae (Caetano et al., 2002).

Throughout evolution, there has been a gradual reduction of the first pair of legs of insects, giving rise to the mandible (Costa-Leonardo, 1978). With its main role related to feeding, the mandible is the jointed appendage of highest development in the Hymenoptera, showing numerous shapes and functions, and reaching its highest level of complexity in ants (Hölldobler and Wilson, 1990). The main use of the mandibles is mechanical, but many authors also relate it to glandular functions (Caetano et al., 2002) since the mandibular gland (Pavon and Mathias, 2001) and the intramandibular gland (Ribeiro and Caetano, 2000) are responsible for the liberation of numerous exocrine substances.

The intramandibular gland was described for the first time in bees by Nedel (1960) and was initially described in ants by Toledo (1967). According to these authors, the intramandibular gland consisted of unrelated cells inside the mandible that communicated to the exterior through canaliculi. Costa-Leonardo (1978) studied this gland in several groups of bees and in her work she noted the presence of a hypertrophied cuticular epithelium. Ribeiro and Caetano (2000) analyzed this hypertrophied epithelium the ant, Zacryptocerus pusillus, and described the presence of secretory epithelial cells. Schoeters and Billen (1994) made a more general analysis of the intramandibular gland of several species of ant, in which, for these authors, the gland was simply composed by secretory cells inside the mandible.

Due to the difficulties of studying materials with a thick cuticle, most of the works concerning the intramandibular
gland appear superficial in relation to the morphological and histochemical aspects of this gland, with the exception of the study made by Ribeiro and Caetano (2000).

Therefore, the object of this work was to describe the cells present inside the mandible of leaf-cutting ants from the ultrastructural, histochemical, and morphological points of view, with a special focus on the epithelial region of the cuticle and with the final aim of obtaining further information on this gland.

2. Materials and methods

Individuals of all castes (soldier, worker, nurses, male and queen) of the ant, Atta sexdens rubropilosa (Hölldobler and Wilson, 1990), were collected from nests found around the UNESP (Universidade Estadual Paulista), campus at Rio Claro-SP, Brazil. Close attention was paid to the males, in which it was possible to ascertain the age of the individual by assessing the level of chitinization of the wings (Della Lucia, 1993). Once extracted, the mandibles were fixed for histology/histochemistry in a solution of 4% paraformaldehyde and processed for resin inclusion. Afterwards, the material was sectioned to a thickness of 6 μm and the sections were stained using the technique of H.E. for morphology (Junqueira and Junqueira, 1983), Alcian Blue-P.A.S. for carbohydrates (Pearse, 1984), P.A.S.-Bromophenol Blue and Xyliidine Ponceau for the detection of proteins (Pearse, 1984), and Toluidine Blue for the detection of

Fig. 1. (A) Longitudinal section of the queen’s mandible. Three cellular types can be observed: cuticular tegument (Ep), secretory cell (Sc), and adipocytes (Fb). The arrows indicate intracellular canaliculi that had a positive reaction to the P.A.S. test. C, cuticle. Technique of P.A.S.-Alcian Blue. Scale bar: 20 μm. (B) Longitudinal section of the worker’s mandible. This figure shows the well-developed secretory epithelium (Se), in which it is possible to observe the reservoirs (Re). Technique of P.A.S.-Bromophenol Blue. Scale bar: 10 μm. (C) Fat body cells of the queen’s mandible. Note the accumulation of carbohydrates (arrowhead). Technique of P.A.S.-Bromophenol Blue. Scale bar: 20 μm. (D) Longitudinal section of the male’s mandible, in which it is possible to observe the degeneration of the cuticular tegument, appearing filiform and highly vacuolated (arrow). Ep, cuticular epithelium; C, cuticle; Fb, fat body cells. Technique of P.A.S.-Bromophenol Blue. Scale bar: 40 μm.
acidic groups (Junqueira and Junqueira, 1983). The material was analyzed and photographed with a Zeiss photomicroscope.

For the ultrastructural analysis the material was fixed in a solution of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), post-fixed in 1% osmium tetroxide, and contrasted in 2% uranyl acetate and a solution of 10% lead citrate. Due to the hardness of the jaw, spurr resin was used, according to technique of Fengshan and Peterson (2000). Sections were obtained using an ultramicrotome collected on copper meshes, and analyzed and photographed with Philips CM 100 (UNESP-Rio Claro/SP) and a Zeiss EM 900 (ESALQ-Piracicaba/SP) transmission electron microscope.

Fig. 2. (A) General view of a transversal section of the queen’s mandible. The contact between the secretory cells (Sc) and the secretory epithelium (Se) is readily observed. It is also possible to observe the intracellular reservoir of the secretory cells (arrow) and the chain of fat body cells (Fb). C, cuticle. Technique of Hematoxilin-Eosin. Scale bar: 46 μm. (B) Longitudinal section of the soldier’s mandible, showing secretory cells (Sc) under the cuticular tegument (Ep). Note the number of reservoirs (arrow). C, cuticle. Technique of Hematoxilin-Eosin. Scale bar: 20 μm. (C) Longitudinal section of the gardener’s mandible showing a hypertrophied cuticular tegument (EP). C, cuticle; H, hemocytes. Technique of Hematoxilin-Eosin. Scale bar: 20 μm. (D) Longitudinal section of the soldier’s mandible presenting a secretory epithelium (Se) with reservoir (Re) and the intracuticular ducts (*). C, cuticle. Technique of Hematoxilin-Eosin. Scale bar: 20 μm. (E) Longitudinal section of the apical region of the soldier’s mandible. Note the numerous epithelial secretory cells (Se) in this region and the intracuticular ducts (*). C, cuticle. Technique of Hematoxilin-Eosin. Scale bar: 46 μm. (F) Epithelial secretory cells (Se) opening at the base of the sensory hair (S). Scale bar: 40 μm.
Table 1
Reaction of Xilidine Ponceau (Pearse, 1984)

<table>
<thead>
<tr>
<th>Secretory epithelium</th>
<th>Nuclei ++</th>
<th>Cytoplasm ++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretory cells</td>
<td>Nuclei ++</td>
<td>Cytoplasm ++</td>
</tr>
<tr>
<td>Fat body cells</td>
<td>Nuclei + +</td>
<td>Cytoplasm −</td>
</tr>
</tbody>
</table>

+, +++, +++, +++++ represent the intensity of reaction.

3. Results

3.1. Histology and histochemistry

Inside the mandible we found three well-defined cellular groups that were present in all castes: fat body cells, cells of the cuticular tegument, and secretory cells in the mandible’s lumen (Figs. 1A and 2A).

The fat body cells appear to be organized in clusters, with the clusters mainly found next to the epithelium of the cuticle (Figs. 1A,D and 2A). Due to the accumulation of reserve materials, the shape of these cells and their nuclei varied (Fig. 2A).

The cuticular tegument is composed of a layer of flattened cells that, in certain regions, become hypertrophied (secretory epithelium) (Fig. 2C,D). The secretion exits through intracuticular ducts which open at the base of jointed hairs present at the internal surfaces of the mandibles (Figs. 1B and 2E,F).

The secretory cells are located next to the cuticular tegument, presenting a rounded shape. It may also possible to observe the reservoir of these cells (Figs. 1A and 2A).

Most of the male specimens showed cellular degeneration, which was characterized by the cellular disorganization inside the mandibles. Such disorganization is noted mainly in the epithelial cells, which appear filiform (Fig. 1D).

The histochemical analysis of the mandibular lumen is presented in Tables 1–4.

3.2. Ultrastructure

The cuticular epithelium of flattened shape presents a large amount of rough endoplasmic reticulum and numerous mitochondria (Fig. 3C). In some individuals, the plasma membrane appeared to be liberating a secretion through membrane evaginations at the region next to the cuticle. The cuticular epithelium is secretory and appears hypertrophied due to the presence of an intracellular reservoir, which is noted as long lamellar projections of the plasma membrane (Fig. 3B). In young individuals (males) the microvilli are anchored to the cuticle through plates (according to Chapman, 1998), which are more electron-dense than other regions of cell (Fig. 3A).

During this developmental stage, the cuticular epithelium presents a large amount of rough endoplasmic reticulum and ribosomes. It was possible to observe microtubules next to the plasma membrane (Fig. 3A).

The secretion produced by the secretory epithelial cells, and also by the secretory cells, is excreted through ducts that traverse through the cuticle; these ducts are internally coated by an epithelium consisting of a single cellular layer (Fig. 4B).

The secretory cells possess large amounts of rough endoplasmic reticulum, Golgi complexes, and mitochondria. They present an intracellular reservoir with numerous evaginations; this reservoir opens to the exterior by means of canaliculi (Fig. 4A).

4. Discussion

In this work we have described three types of cells present inside the mandible. These are: the cells of the secretory epithelium, the secretory cells located next to the epithelium, and the fat bodies cells.

The fat body cells function in the synthesis and/or storage of carbohydrates, lipids, and proteins, being one of the main cellular groups responsible for intermediary metabolism (Chapman, 1998). The close relationship between the fat bodies and the cells of the cuticular epithelium and secretory cells suggests that the former might serve as metabolic support for the production of the secretion of the intramandibular gland. Ribeiro and Caetano (2000) describe the presence of secretory cells with microvilli in the mandible’s lumen, located next to the fat bodies cells. We were not able to find this cellular group. Costa-Leonardo (1978) mentions the presence of fat body cells and suggests that their function would be to fill the mandible.

The secretory cells that are located next to the cuticular tegument are classified as type 3 secretory cells according to the classification proposed by Noirot and Quennedey (1991).

Table 2
Reaction of P. A. S.-Bromofenol Blue (Pearse, 1984)

<table>
<thead>
<tr>
<th>Secretory epithelium</th>
<th>Nuclei −</th>
<th>Cytoplasm +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretory cells</td>
<td>Nuclei +</td>
<td>Cytoplasm +</td>
</tr>
<tr>
<td>Fat body cells</td>
<td>Nuclei −</td>
<td>Cytoplasm −</td>
</tr>
</tbody>
</table>

+, +++, +++, +++++ represent the intensity of reaction.

Table 3
Reaction of P.A.S.-Alcian Blue (Pearse, 1984)

<table>
<thead>
<tr>
<th>Epithelial cell</th>
<th>Nuclei ++</th>
<th>Cytoplasm +</th>
</tr>
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<tbody>
<tr>
<td>Secretory cells</td>
<td>Nuclei −</td>
<td>Cytoplasm −</td>
</tr>
<tr>
<td>Fat body cells</td>
<td>Nuclei −</td>
<td>Cytoplasm −</td>
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+, +++, +++, +++++ represent the intensity of reaction.

Table 4
Reaction of Toluidine blue (Junqueira and Junqueira, 1983)

<table>
<thead>
<tr>
<th>Epithelial cell</th>
<th>Nuclei + +</th>
<th>Cytoplasm +</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat body cells</td>
<td>Nuclei + +</td>
<td>Cytoplasm +</td>
</tr>
</tbody>
</table>

+, +++, +++, +++++ represent the intensity of reaction.
This type of secretory cell is always found associated with the cuticle, but in this case it appears under the cuticular tegument. The production of this group of cells might vary from the production of pheromones of attraction and repulsion (Quennedey, 1998) to the production of wax (King and Akai, 1982). The histochemical tests resulted in a stronger positive reaction to the tests for P.A.S. (Table 2) and Xylidine Ponceau (Table 1). This suggests that the secretion liberated by these cells is related to the production of enzymes, due to its positive reaction for proteins, as also with the production of mucus. Schoeters and Billen (1994) described these cells as being the main cells responsible for production of the secretion of the intramandibular gland; however, they did not hypothesize about the kind of secretion. We disagree with these authors, since it cannot be affirmed that these secretory cells are directly related to the liberation of all the secretion of the intramandibular gland. Our proposition is reinforced by the fact that other cells with characteristics of glandular cells are found in the mandible’s lumen, fact that was also noted by Ribeiro and Caetano (2000). Contrary to Schoeters and Billen (1994), we propose that the secretory cells present in the cuticular epithelium of the mandible are the main cellular

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**Fig. 3.** (A) Transmission electron photomicrograph of the cells of the cuticular tegument of the male’s mandible (age of up to 48 h). Note the large number of ribosomes (rb) and the anchoring region of the microvilli (double arrows) in the cuticle (C). mi, microtubules. Scale bar 3 μm. (B) Photograph taken under the transmission electron microscope of a queen’s epithelial secretory cell. Note the secretion (S) accumulated in the reservoir of the cell (Re) which is located in the cuticular tegument. The figure show long evaginations of the plasma membrane (mp), and the electron-lucid vesicles (v) present inside and outside the reservoir. Note that some of these vesicles are similar to myelinic bodies (mf) at the initial stages of formation. gc, Golgi complex; m, mitochondria. Scale bar: 1.6 μm. (C) Epithelial cell of the male’s cuticle. This figure shows the contact between a cell of the secretory region of the cuticular tegument (Ep) and the more internal secretory cells (Sc). Electron-dense bodies are forming next to the nucleus (b). m, mitochondria. Scale bar: 1.7 μm.
group responsible for the liberation of the intramandibular gland’s secretion. This hypothesis is confirmed by the fact that this cellular group is only found associated with the cuticle of the internal surface of the mandible, this description having been made by Ribeiro and Caetano (2000) in the ant Zacryptocerus pusillus. Another characteristic of the secretory epithelial cells is that they can be classified as type 1 cells as well as type 3 cells (secretory epithelium) according to the classification proposed by Noirot and Quennedey (1991). This observation might be related to the age of the individual, since in younger individuals the function of the epithelium is the formation of the cuticle, but, after reaching a certain age, this epithelium might form an intracellular reservoir that produces and liberates its secretion through another cell present inside the canaliculi. In adult individuals, this epithelium plays a role in the maintenance of the cuticle and in some cases part of it can hypertrophy and acquire glandular characteristics temporarily or definitively. The case of the wax gland of bees is a typical example of this phenomenon (Chapman, 1998).

Even though the mandible possesses an internal nerve, we did not find a direct relationship between this intramandibular nerve and the liberation of the gland’s secretion. According to Billen and Morgan (1998), the liberation of the secretion of exocrine glands might be accomplished through neural or hormonal stimuli. Thus, since we did not find neurosecretory vesicles or a point of contact between nerve cells and epithelial cells, it is possible that the secretion process of the intramandibular gland is controlled by hormones transported through the haemolymph. The nerve present inside the mandible would be associated with the sensory hairs of the mandible.

As a result of this work it was possible to conclude that the intramandibular gland is composed of at least two cellular groups involved in secretion: the secretory cells next to the epithelium that are located in the mandible’s lumen, and the secretory epithelium. The association of these cells with the fat body cells leads us to believe that the fat body cells play an important role in the maintenance of the secretory process.

Because of the multiple functions of the mandible, it is difficult to define a specific role for the intramandibular gland. Given the degeneration of the cells present inside the male’s mandible, we may assume that in these individuals the secretion probably is not only related to digestive process, since males die immediately after the nuptial flight. Due to the fact that the secretory epithelial cells and the secretory cells are on a single face of the mandible, we believe that there is a direct relationship between the secretion and feeding, since the secretion produced is directed towards the internal face of the mandible, in direct contact with the meal. Because the secretion of the epithelial cells is released at the base of the sensorial hairs present on the mandible’s surface, the liberation of the secretion might also be related to the mechanical aspects of food capture.

Finally, due to the difficulties inherent in performing bioassays followed immediately by the processing of the material, we cannot be certain about the function of this gland, and thus its real role remains unknown.

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