The midgut of Cephalotes ants (Formicidae: Myrmicinae): Ultrastructure of the epithelium and symbiotic bacteria

Murillo L. Bution a,∗, F.H. Caetano b

a Universidade Estadual de Campinas (UNICAMP), Departamento de Genética, Evolução e Bioagentes do Instituto de Biologia, Campinas S.P. 13083-970, Brazil
b Universidade Estadual Paulista (UNESP), Departamento de Biologia, Rio Claro, S.P. 13506-900, Brazil

ARTICLE INFO

Article history:
Received 1 December 2009
Received in revised form 2 February 2010
Accepted 8 February 2010

Keywords:
Midgut
Gram-negative bacteria
Spherocrystals
Ants

ABSTRACT

The ultrastructural analysis of the midgut of Cephalotes atratus, C. clypeatus, and C. pusillus revealed that the midgut epithelium lays on a basal lamina and is composed basically of three cell types: digestive cells, regenerative cells, and goblet cells. In these ants, the rough endoplasmic reticulum, in addition to producing digestive enzymes, is involved in the formation of concretions and ion storage in specialized vacuoles present in the midgut. These concretions are spherocrystals and may contribute to stabilize the pH and to maintain symbiotic bacteria found between microvilli. The ultrastructure analysis of these bacteria revealed the presence of a double envelope typical of gram-negative bacteria. For the three species examined, the ultrastructure similarities are conspicuous, suggesting that this may be the pattern for the genus Cephalotes. Details of the relationship between bacteria and microvilli were examined.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

The midgut of ants as well as of other insects derives from the endoderm, and therefore is not lined by a cuticle. Instead, the peritrophic matrix covers and protects the midgut against injuries (mechanical or chemical) that might occur during the passage of the food bolus (Chapman, 1975; Caetano, 1984; Caetano, 1988; Lehane and Billingsley, 1996). In addition, the peritrophic matrix compartmentalizes the midgut, allowing an efficient circulation of enzymes in the endo and ectoperitrophic compartments (Terra and Ferreira, 1994; Terra et al., 1996; Terra, 2001).

Caetano et al. (1994a,b) have shown that the secretion vesicles produced in the midgut of Pachycondyla striata cross the peritrophic matrix, and begin to disintegrate as they reach the endoperitrophic space.

The midgut of insects consists of a single layer of epithelium that lays on a continuous basal lamina (Wigglesworth, 1974; Chapman, 1975; Caetano, 1984; Caetano, 1988; Lehane and Billingsley, 1996). This epithelium is composed of digestive cells and regenerative cells, but in some cases, other cell types, such as endocrine cells and goblet cells, may be present. These differentiated cells are involved in one or more processes, such as secretion, digestion, absorption, and storage of several compounds (Snodgrass, 1935; Wigglesworth, 1974; Chapman, 1975; Martoja and Ballan-Dufarnciais, 1984).

Externally surrounding the basal membrane, circular and longitudinal muscle fibers are responsible for peristaltic movements in this region of the digestive tract (Snodgrass, 1935; Caetano, 1984; Caetano, 1988; Arab and Caetano, 2001).

Symbiotic bacteria have been described in the midgut of several ant species, including species of the genus Camponotus (Schröder et al., 1996). These bacteria are present inside specialized cells called bacteriocytes in the midgut.

These bacteria, first observed by Blochmann (1882), are phylogenetically very close to endosymbiotic bacteria of insects such as Buchnera aphidicola in aphids, Wigglesworthia glossinidia in the tsetse fly Glossina palpalis (Baumann et al., 1995; Schröder et al., 1996; Chen et al., 1999). Caetano et al. (2009) described the presence of bacteria, called endocytobionts, inside epithelial cells of the midgut of Odontomachus bauri (Caetano and Cruz-Landim, 1985; Caetano, 1989; Roche and Wheeler, 1997; Bution et al., 2006). These authors suggested that these bacteria participate in the digestion of food and thus act as symbionts as they open new possibilities for ants to explore food resources from the environment.

Bacteria in the midgut have also been described in Dolichoderus (=Monacis) bispinosus and in ants of the Cephalotini tribe. These authors suggest that the presence of a symbiotic interaction between bacteria and their ant hosts.

In this study, the structural characteristics of the midgut of Cephalotes atratus, C. clypeatus and C. pusillus were analyzed and compared, as well as the differences in the epithelium and the content of this portion of the digestive tract, as a contribution to understand the relationships between bacteria and their ant hosts.
2. Materials and methods

2.1. Animals

Large workers of *Cephalotes pusillus* and workers of *C. clypeatus* were collected from trees located at the UNESP/Rio Claro campus, while the larger workers of *C. atratus* were collected from trees located at the margins of Jacaré-pepira River, in Brotas city, State of São Paulo/Brazil.

2.2. Transmission electron microscopy (TEM)

The midgut of *C. atratus*, *C. pusillus* and *C. clypeatus* were carefully removed from the abdomen directly in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and fixed for 2 h. Post-fixation was carried out in 1% osmium tetroxide in the same buffer. En bloc staining with 2% uranyl acetate was followed by a series of ethanol dehydration steps. Midguts were then embedded in Eppon resin and sectioned with a Leica ultramicrotome. Ultra-thin sections were stained with uranyl acetate and led and photographed with a CM 100 Phillips TEM at 80 kV.

3. Results and discussion

The midgut epithelium of *Cephalotes atratus*, *C. clypeatus*, and *C. pusillus* lays on a continuous and thick basal lamina and consists of basically three cell types: digestive or columnar cells (that can be divided into 2 or 3 subtypes, depending on ultrastructural characteristics, which reflect their roles), regenerative cells, and goblet cells. These results are in agreement with those reported by *Caetano and Cruz-Landim* (1983) for *Camponotus arboresus*.

*Caetano et al.* (1994a,b) described goblet cells as clusters of four cells that form a cup-like structure with a wide base and a deep invagination at the apical area in the ant *Pachycondyla striata*. The formation of this “cell” is the result of a depression of the apical membrane, and the cavity formed has many long microvilli, which were clearly observed with the technique used (Figs. 1 and 2).

The structural characteristics of goblet cells reflect their role in ionic regulation, promoting the exchange of K⁺, Na⁺, and H⁺ between the hemolymph and the lumen of the midgut. *King and Akai* (1984) also suggested that goblet cells are involved in the active transport of calcium ions from adjacent columnar cells. These compounds are later transported to the lumen of the midgut. Thus, the excess of these substances in the hemolymph may be regulated in part by cells and in part by the Malpighian tubules (Chapman, 1975).

Regenerative cells in various developmental stages are located near the basal lamina. The cytoplasm has few organelles, and the nucleus is irregular and large in relation to the cytoplasm. The dispersed chromatin suggests a high metabolic level (Figs. 3–8). Similar characteristics have also been reported for the regenerative cells of *S. saevissima* (Arab and Caetano, 2001). Thus since early developmental stages, regenerative cells present characteristics that will define their roles at maturity—several mitochondria, well-developed rough endoplasmic reticulum, and irregular-shaped nucleus.

Digestive or columnar cells are the most abundant cell type found in the midgut epithelium. They exhibit several in folds in the basal plasma membrane that comprise the basal labyrinth interposed between many mitochondria (Figs. 9–14), indicating intense activity. According to *King and Akai* (1984) and *Caetano and Cruz-Landim* (1983), the ultrastructure of these cells varies and depends mainly on the multiple roles they may have during their life cycle. These cells may be involved in the secretion of enzymes, mucopolysaccharide, absorption and storage of organic and inorganic products. Figs. 4–9, 15–17 illustrate the characteristics that define their activities.

In *C. atratus*, *C. clypeatus*, and *C. pusillus*, as well as in other insects described by *King and Akai* (1984), columnar cells also may excrete and accumulate mineral concretions in multilayers called spherocrystals (Figs. 9 and 10). These spherocrystals consists of mainly cations (Ca⁺⁺ and Mg⁺⁺), phosphates, carbonates, and chlorides absorbed from food (Wigglesworth, 1974; *Caetano and Cruz-Landim*, 1983; *King and Akai*, 1984; Arab and Caetano, 2001).

The cytoplasm of columnar cells in these ants has a well-developed rough endoplasmic reticulum (RER) and the Golgi complex nearby (Figs. 11–13). These characteristics suggest the synthesis of proteins of digestive enzymes. In *C. atratus*, *C. clypeatus*, and *C. pusillus*, the RER is also involved in the storage of ions in specialized vacuoles present in the midgut. These concretions originate spherocrystals (Figs. 9–11).

In *Formica polyctena*, spherocrystals are secreted with part of the apical cytoplasm (Jeantet, 1971). Similar characteristics were found in *C. atratus*, *C. clypeatus*, and *C. pusillus*, where part of the spherocrystals released into the lumen originates from apocrine columnar cells.

The presence of spherocrystals in the lumen of the midgut of the ants examined might contribute to pH stabilization and in turn,
the maintenance of bacteria in this region of the digestive tract (Fig. 5). This is supported by the empty spherocrystals observed in the lumen, indicating that the contents were partially or totally removed, thus contributing to alter the pH of the region, as proposed in our study.

One of the most interesting roles played by columnar cells was described by Jeantet (1971). This study showed that when ants are contaminated with toxic metals, several lysosomes form in the apical region of columnar cells, which along with spherocrystals, accumulate metals and ensure detoxification.

Multivesicular bodies were observed in the cytoplasm of columnar cells (Fig. 14). They are vacuoles containing substances from two distinct origins, pinocytic vesicles originated near the surface of the plasma membrane and primary lysosomes of the Golgi complex (King and Akai, 1984). Their roles are probably recycling of the plasma membrane and digestion of intracellular proteins. Because
Figs. 9–14. Electron micrographs of the midgut epithelium of C. clypeatus (Figs. 9 and 11) and C. pusillus (Fig. 10) showing columnar cells involved in the production, storage, and secretion of spherocrystals (Sc). In this species, spherocrystals are produced by the rough endoplasmic reticulum (Rer). Figs. 12 and 13 Details of the cytoplasm of columnar cells of the midgut of C. clypeatus and C. pusillus, respectively, showing large amounts of rough endoplasmic reticulum (Rer) involved in the concretion of spherocrystals and in the synthesis of digestive enzymes, as a result of its location adjacent to the Golgi Complex (G). Polyribosomes (Prb). Fig. 14 Details of the cytoplasm of the columnar cells of the midgut of C. pusillus showing multivesicular bodies (Mvb) in early stages of degeneration near a mitochondrion (Mit) and lipid droplets (Li).

Multivesicular bodies are involved in the recycling of membranes, they are associated mainly with secretory cells, as a result of the large demand to replace the membranes used in surface invaginations, in the formation of vesicles, and in reticular systems (King and Akai, 1984).

In the ectoperitrophic space, several bacteria are observed between microvilli (Figs. 1, 4, 5, 15, 18 and 19). This is a strategic location as it avoids the contact of bacteria with digestive enzymes, since secretion vesicles circulate with intact membranes throughout the ectoperitrophic space until they cross the peritrophic matrix. As they reach the endoperitrophic space, the vesicles begin to show signs of disintegration as described by Caetano et al. (1994a,b).

The ultrastructure analysis of these bacteria revealed the presence of a double envelope typical of gram-negative bacteria, according to Caldwell (1995) (Figs. 18–23). Many of these bacteria were observed reproducing, thus clearly adapted to this region of the digestive tract (Figs. 18, 20, 23). Caldwell (1995) reported that the double envelope of gram-negative bacteria have distinct structural and functional properties from those found in the cell wall of gram-positive bacteria described in the ileum of C. atratus, C. clypeatus, and C. pusillus (Jaffé et al., 2001). The cell wall of gram-
positive bacteria is chemically less complex than the cell envelope of gram-negative bacteria and is composed basically of murein (peptidoglycan), which is in contact with the environment. Consequently, hydrolytic enzymes, such as lysozyme, are more effective against gram-positive bacteria.

There are indications that the presence of digestive enzymes, such as lysozyme in the midgut of *C. atratus*, *C. clypeatus*, and *C. pusillus*, might be a determinant factor for the location of distinct types of symbiotic bacteria throughout the digestive tract. This hypothesis is supported by the presence of gram-negative bacteria only in the midgut and gram-positive bacteria only in the ileum of these ants (Bution and Caetano, 2008). Because of the composition of the cell wall, gram-positive bacteria would be easily lysed by the action of enzymes in the midgut (Lehane and Billingsley, 1996). This suggests that these enzymes do not remain active throughout the digestive tract, but rather only in the midgut.

The space between the external and internal plasma membrane of gram-negative bacteria contains a rare and unique fluid region, the periplasm (Caldwell, 1995). This author describes the periplasm as responsible for a large part of the physiological events that are critical for the survival of these bacteria. The periplasmic space also has membrane receptors and other proteins that mediate the transport of substances through the bacterial envelope. The periplasm can be easily observed in the bacteria present in the midgut of *C. atratus*, *C. clypeatus*, and *C. pusillus* (Figs. 20–23).

Jones (1983) reported that organisms with nutritional limitations due to low nutritional content of the main food resource may adopt mechanisms that allow the optimization of this resource. Thus the mechanism adopted by these ants, similar to other insects, such as termites, cockroaches and beetles, is the symbiosis established with gut microorganisms.

In general, ants feed preferentially on fluids composed mainly of polysaccharides. The presence of symbiotic microorganisms in the midgut of *C. atratus*, *C. clypeatus*, and *C. pusillus* may supplement their diets.

For the three species examined, the ultrastructure similarities are conspicuous, suggesting that this may be the pattern for the genus *Cephalotes*. 
Figs. 18–23. Electron micrographs of the ectoperitrophic space of the midgut of C. pusillus, showing ultrastructural details of the bacteria between microvilli. The internal membrane (Im) and external membrane (Em) are typical of the envelope of gram-negative bacteria. Between these membranes of the bacterial envelope is the periplasm (P). Some bacteria are reproducing, as shown by the presence of division septum (Ds) and two nucleoids (Nuc).

References


