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Elemental composition of biomineralized amorphous mineral granules isolated from ants: Correlation with ingested mineral particles from the soil

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ABSTRACT

Amorphous mineral granules are formed by concentric mineral layers containing polyphosphate, pyrophosphate and/or orthophosphate and several metallic cations such as Mg^{2+}, Ca^{2+}, K^{+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+}. In this work, we analyzed amorphous mineral granules isolated from the ant species *Camponotus abdominalis*, *Camponotus* sp., *Acromyrmex subterraneus* and *Pachycondyla marginata* by energy-dispersive X-ray analysis. The elemental composition of the granules was compared to that of mineral particles, probably soil particles, to access the influence of the environment and of specific characteristics of each ant species in the elemental composition of the amorphous mineral granules. Both the granules and mineral particles presented Mg, Ca, Fe, and Zn in the four species. Additionally, Al tended to be present in both (or none) of the two types of material in a given ant species, suggesting that the aluminum found in the amorphous mineral granules could be derived from ingested soil particles. On the other hand, Sr was found in the amorphous mineral granules of some of the studied ant species, but not in the mineral particles. The fact that 3/4 of the elements found in the granules were found also in the mineral particles suggests that the mineral composition of the soil plays a fundamental role in the accumulation of some elements in the amorphous mineral granules of ants. These results suggest a major role of soil particles as a source of micronutrients for the four ant species.

1. Introduction

It is well documented that several invertebrate tissues present biomineralized metal-containing amorphous granules. In crustaceans and insects, amorphous calcium phosphates have been found in granules or concretions composed by alternated concentric layers of higher and lower mineral content (Ballan-Dufrançais, 2002; Brown, 1982; Coombs and George, 1978; Corrêa Jr. et al., 2009; Lipovšek Delakorda et al., 2009; Lowenstam, 1981; Martoja and Ballan-Dufrançais, 1984; Martoja et al., 1975; Simkiss and Wilbur, 1989). Orthophosphate and pyrophosphate are the main P-containing components in the amorphous mineral granules (AMG) of the mollusk *Helix aspersa* and the crab *Carcinus maenas* (Lee et al., 1995). In addition to orthophosphate and pyrophosphate, small amounts of glucose-6-phosphate is also found in the AMG of the crab *Ucides cordatus* (Corrêa Jr. et al., 2009). In normal conditions, calcium phosphates in solution would transform into a crystalline phase, but the presence of ions such as Mg^{2+}, Zn^{2+}, and di- and triphosphates may inhibit this transformation (Simkiss and Wilbur, 1989; Simkiss, 1993). In crustaceans, mollusks and insects, it is considered that AMG work in both metal storage (Brown, 1982; Lipovšek Delakorda et al., 2009; Simkiss and Wilbur, 1989) and detoxification processes (Ballan-Dufrançais, 2002; Brown, 1982; Grześ, 2010a; Jeantet et al., 1977; Simkiss and Wilbur, 1989). The functions of these granules in storage and detoxification of metallic elements in invertebrate tissues is related to their high capability to incorporate foreign ions as compared to their crystalline equivalents (Simkiss and Wilbur, 1989).

In adult worker ants, AMG are found in the endoplasmic reticulum–Golgi complex of cells from the midgut, malpighian tubules and the fat body. Among these sites, the midgut shows the highest concentrations of the AMG (Jeantet et al., 1977; Ballan-Dufrançais, 2002). The midgut, the malpighian tubules and the hindgut were shown to contain the highest concentrations of Pb, Cd, Cu, Zn, Fe, and Mn in the ants *Formica pratensis*, *Formica polyctena* and *Camponotus ligniperda* collected in a metal-polluted site (Rabitsch, 1997b), whereas the gut was shown to be the main site for storage of Mn, Cu and Zn, and the fat body the main site

Abbreviation: AMG, amorphous mineral granules.

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for Fe storage in *Crematogaster scutellaris* (Gramigni et al., 2011). Accordingly, AMG from the midgut of *F. polyctena* (Jeantet et al., 1974, 1977) and *Formica rufa* (Ballan-Dufrançais et al., 1971) usually contain P, Ca, Mg, K, Mn, Fe, and Zn. In addition, small amounts of Ba have been found in the AMG from *F. Polyctena* (Jeantet et al., 1974, 1977). The AMG from the malpighian tubules of *F. polyctena* contained Ca, Mg, K, and Mn and those of the fat body contained Ca, Mg, and K (Jeantet et al., 1977). Ants exposed to V, Co, Cu, Pd, Cd, Sn, Sb, Ba, Pt, Hg, and Pb under laboratory conditions accumulate these metals mainly in the AMG of the midgut epithelium. The survival to high concentrations of these elements shows that they are very resistant to them (Jeantet et al., 1977; Ballan-Dufrançais, 2002).

In this work, we analyzed both AMG and soil particles isolated from *Camponotus abdominalis*, *Camponotus* sp., *Acromyrmex subterraneus* and *Pachycondyla marginata* worker ants to access the influence of the environment and of species-specific differences on the elemental composition of the AMG.

2. Materials and methods

More than 200 individuals of *C. abdominalis*, *Camponotus* sp. and *A. subterraneus* were collected in the ground in Rio de Janeiro city (RJ), Brazil (22°56′S, 43°12′W) in August 1994. *P. marginata* was collected in "Reserva Santa Genebra" (22°49′S, 47°06′W), in Campinas (SP), Brazil, between October 1992 and September 1993. They were maintained in 70% ethanol solution until the start of the digestion treatment.

For a specific search of AMG in the ant bodies, we processed the ants separately in three parts: head, thorax and abdomen. Antennae and legs were discarded. The digestion process started with maceration in the presence of 5% sodium hypochlorite solution and further visual separation of cuticle, when possible, under a stereomicroscope. Afterwards, approximately equal amounts of each isolated sample were placed in 1.5 ml polypropylene tubes and more NaOCl solution was added until filling half of the volume of the tubes. The samples were left overnight in the refrigerator. Then, the samples were centrifuged (5000 rpm by 8 min), the supernatant was discarded, new NaOCl solution was added and the pellet was resuspended both manually and with ultrasonic vibration for 15 min. This process was repeated until the pellet did not change in color after new cycles of treatment, indicating that most of the organic matter had been digested. Then, the pellet was washed in distilled water using the same routine of washing–pelleting–resuspending until the NaOCl had been removed. One drop of this sample was deposited onto Formvar-covered electron microscopy copper grids, dried with filter paper, and observed in a Zeiss CEM902 or a Jeol 1200 EX transmission electron microscopes. Energy-dispersive X-ray (EDX) analysis was performed in a Jeol 1200 EX transmission electron microscope equipped with a Noran EDS system.

3. Results

We found AMG only in the abdomen isolates, as expected, since all the organs described to contain them (midgut, malpighian tubules and the fat body) are present in this tagma of the ant bodies (Ballan-Dufrançais, 2002; Jeantet et al., 1977). These structures were formed by concentric layers with different sizes and electron densities (Fig. 1). X-ray microanalysis showed that all AMG contained P and Ca as characteristic elements (Fig. 2 and Table 1) and also Mg, Si, Fe and Zn in different proportions (Fig. 2 and Table 1). Diffuse rings were obtained from the AMG by selected area electron diffraction showing their amorphous nature (not shown), which was expected for granules containing Mg and Zn besides Ca and P. S was found in the AMG of *A. subterraneus* and *P. marginata* but not

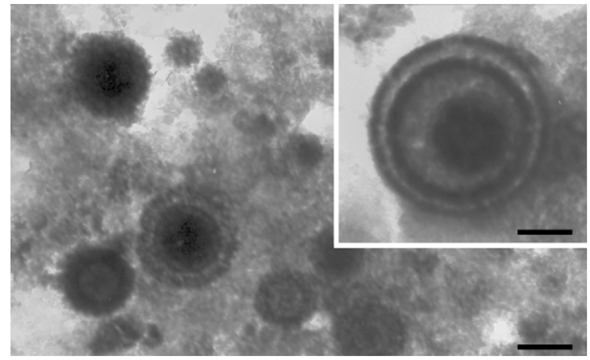

Fig. 1. Transmission electron microscopy image of amorphous mineral granules (AMG) and mineral particles from the abdomen of *Acromyrmex subterraneus*. Bar = 1 μm. Inset: detail of an AMG isolated from *Camponotus* sp. showing the typical concentric layers. Bar = 0.5 μm.

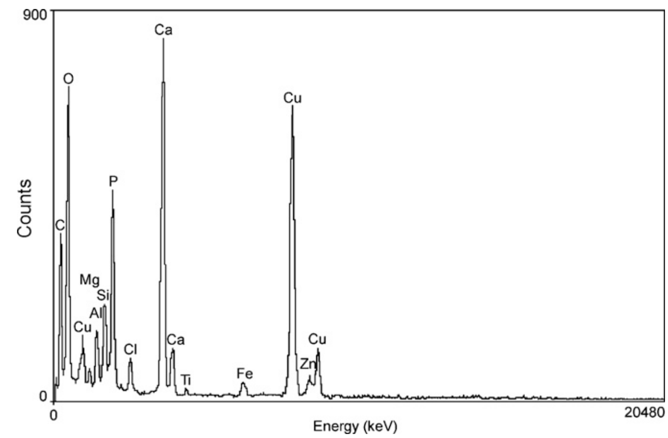

Fig. 2. Typical energy dispersive X-ray spectrum of an AMG isolated from *Camponotus abdominalis*. This granule contains Mg, Al, Si, P, Cl, Ca, Ti, Fe, and Zn. Cu peaks are derived from the supporting grid.

in the two *Camponotus* species studied. Cl was found in all AMG and could be derived from the NaOCl used in the isolation procedure.

Dispersed particles isolated together with the AMG presented various types of diffraction patterns (not shown) showing that most of them were crystalline. They were easily distinguished from the AMG as they had irregular shapes and dimensions (Fig. 3) whereas the AMG were spherical (Fig. 1). These mineral particles showed Ca and Si as major elements and also high amounts of P in *C. abdominalis* and *Camponotus* sp. and Al in *A. subterraneus* and *P. marginata* (Fig. 4). Both the crystalline nature and the high content of silicon

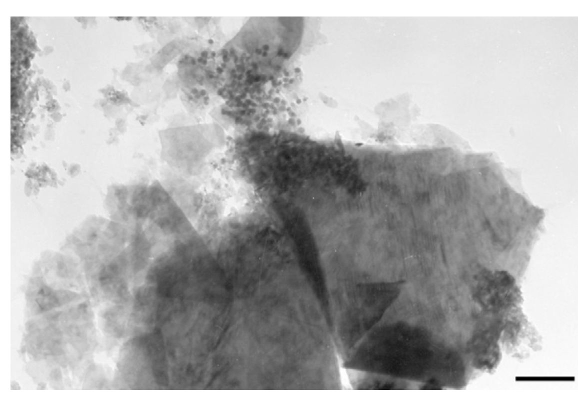

Fig. 3. Transmission electron micrograph of mineral particles isolated from *A. subterraneus*. Observe the irregular shape of the particles. Bar = 1 μm.