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A new intracellular pathway of haem detoxification in the midgut of the cattle tick *Boophilus microplus*: aggregation inside a specialized organelle, the hemosome

Flavio Alves Lara[1], Ulysses Lins[2], Gabriela Paiva-Silva[1], Igor C. Almeida[3], Cláudia M. Braga[4], Flávio C. Miguens[5], Pedro L. Oliveira[1] and Marílvia Dansa-Petretski[5,*]

[1]*Departamento de Bioquímica Médica, ICB* and [2]*Departamento de Microbiologia Geral, IMPPG, Universidade Federal do Rio de Janeiro, Brazil,* [3]*Departamento de Parasitologia, ICB, Universidade de São Paulo, Brazil,* [4]*Divisão de Química, Petrobrás/CENPES, Rio de Janeiro, Brazil* and [5]*Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Brazil*

*Author for correspondence (e-mail: marilvia@uenf.br)

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Summary

The hard tick *Boophilus microplus* ingests large volumes of cattle blood, as much as 100 times its own mass before feeding. Huge amounts of haem are produced during haemoglobin digestion, which takes place inside acidic lysosomal-type vacuoles of the digest cells of the midgut. Haem is a promoter of free radical formation, so haemoglobin digestion poses an intense oxidative challenge to this animal.

In the present study we followed the fate of the haem derived from haemoglobin hydrolysis in the digest cells of the midgut of fully engorged tick females. The tick does not synthesize haem, so during the initial phase of blood digestion, absorption is the major route taken by the haem, which is transferred from the digest cells to the tick haemocoel. After this absorptive period of a few days, most of the haem produced upon haemoglobin degradation is accumulated in the interior of a specialized, membrane-delimited, organelle of the digest cell, herein called hemosome. Haem accounts for 90% of the hemosome mass and is concentrated in the core of this structure, appearing as a compact, non-crystalline aggregate of iron protoporphyrin IX without covalent modifications. The unusual FTIR spectrum of this aggregate suggests that lateral propionate chains are involved in the association of haem molecules with other components of the hemosome, which it is proposed is a major haem detoxification mechanism in this blood-sucking arthropod.

Key words: tick, haemozoin, haem, cattle tick, *Boophilus microplus.*

Introduction

Haem is found in all living cells as the prosthetic group of a wide range of proteins such as cytochromes, haemoglobins and peroxidases. Nevertheless, the potential toxicity of the haem has been extensively demonstrated (Aft and Mueller, 1983; Vincent et al., 1988; Schmitt et al., 1993) and is often connected with its action as a catalyst for the formation of oxygen radicals. Haem can also associate to phospholipid membranes and make them leaky, by physically disturbing the bilayer structure (Schmitt et al., 1993).

Organisms that feed on vertebrate haemoglobin face a very special situation as digestion results in the production of remarkably high amounts of haem. Biocrystallization of haem into an insoluble aggregate called haemozoin is a major defence mechanism that makes it possible for several organisms to feed on blood. The structure of haemozoin has recently been resolved (Pagola et al., 2000) and its presence, originally thought to be restricted to the malaria parasite, has now been detected in *Rhodnius prolixus*, a blood-sucking insect (Oliveira et al., 1999), and in the blood fluke *Schistosoma mansoni* (Oliveira et al., 2000). Haem aggregation occurs either in an intracellular digestive vacuole where the haemoglobin molecule is hydrolyzed, as in *Plasmodium* (Goldberg et al., 1990; Slater et al., 1991), or extracellularly, as in *Rhodnius* and *Schistosoma* (Oliveira et al., 1999, 2000).

After hatching, the *Boophilus microplus* larvae find their vertebrate host. During the following 3 weeks, the tick larva feeds on small amounts of blood, and after maturation, over a period slightly longer than 1 day, the adult female ingests blood equivalent to approximately 100 times its own body mass. Being a single-host tick, the engorged female drops from the bovine host and dies approximately 1 month later. Most of the digestion takes place over a few days following the meal, in parallel with the development of a large number of eggs. In insects, digestion takes place at the midgut lumen, but ticks have developed a differentiated cell lineage that phagocytoses the blood meal, so digestion is thought to be essentially

intracellular, accomplished by lysosomal hydrolytic enzymes in the interior of an acidic vacuole (Gough and Kemp, 1995; Mendiola et al., 1996). In the present work, we have studied the fate of haem in the midgut of *Boophilus microplus* and show that, after hydrolysis of haemoglobin by the digest cells, the major haem detoxification mechanism is its sequestration into a non-crystalline aggregate that is different from haemozoin. This aggregate is formed and stored in a particular type of intracellular vesicle that is distinct from the digestive vesicle.

Materials and methods

Animals

Boophilus microplus Cannestrini 1887 were obtained from a colony maintained at the Faculdade de Veterinária of Universidade Federal do Rio Grande do Sul, Brazil. *Boophilus microplus* of the Porto Alegre strain, free of *Babesia* spp., were reared on calves obtained from a tick-free area. Engorged adult females were kept in Petri dishes at 28°C and 80% relative humidity until use.

Histochemistry

Engorged females were dissected in modified Karnovsky's fixative (2.5% glutaraldehyde, 4% paraformaldehyde, 100 mmol l^{-1} $CaCl_2$ and 0.1 mol l^{-1} sodium cacodylate buffer, pH 7.3). Tissues were transferred to fresh fixative and kept at 4°C for 12 h. Segments of the anterior portion of the midgut were then washed in 0.1 mol l^{-1} sodium cacodylate buffer, pH 7.3. The tissues were dehydrated in ethanol and embedded in Historesin (Leica, Wetzlar, Germany). Semi-thin (5 μm) sections were observed by differential interference contrast (DIC) microscopy (Axioplan, Zeiss, Esslingen, Germany).

Transmission electron microscopy

The tissues were fixed as above and post-fixed in buffered 1% osmium tetroxide, 0.8% potassium ferrocyanide at room temperature for 1 h, dehydrated in acetone and embedded in epoxide resin. Ultra-thin sections (70 nm) were stained with uranyl acetate and lead citrate (Reynold's method, as described in Glauert, 1974) and were observed with a Zeiss 900 transmission electron microscope at 80 kV.

Cytochemistry

To locate areas of higher haem concentration, we used the methodology of Graham and Karnovsky (1966), which is based on the capacity of haem to promote oxidation of 3-3-diaminobenzidine (DAB). Midguts were dissected and fixed with 1% glutaraldehyde in 0.1 mol l^{-1} sodium cacodylate buffer, pH 6.5. After several washings in sodium cacodylate buffer, tissues were incubated in 2.5 mmol l^{-1} DAB for 1 h at 37°C, and then transferred to 0.03% hydrogen peroxide for 1 h at 37°C. Controls were performed in the absence of hydrogen peroxide.

Isolation of hemosomes

Engorged females were dissected on the day 10 after a blood meal (ABM) in 0.15 mol l^{-1} NaCl, 20 mmol l^{-1} sodium phosphate buffer, pH 7.2, plus 10 mmol l^{-1} $CaCl_2$. The gut content was centrifuged at 1000 ***g*** for 1 min and the supernatant was discarded. The pellet was washed with the same solution (3×) and further purified by means of a 4.5 ml Percoll–0.2 mol l^{-1} sucrose (9:1 v/v), laid over a cushion of 0.5 ml 60% sucrose and centrifuged at 53,000 ***g*** for 1 h in a Hitachi CB ultracentrifuge (CA, USA).

Haem content and light absorption spectra

Alkaline pyridine-haemochrome derivatives were obtained as described by Falk (1964), and visible absorption spectra were obtained using a Zeiss spectrophotometer model Spekord. Haem content was determined from the reduced minus oxidized spectra of the pyridine alkaline derivative.

HPLC fractionation

High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-10AT (Maryland, USA) device equipped with a diode array detector (SPD-M10A), and fractionation of hemosomes was performed using a Shimadzu CLC-ODS column (15 mm×22 cm). Hemosomes were frozen, thawed, suspended in deionised water and centrifuged at 12 000 ***g*** for 1 min. The pellet, containing the hemosome core, was dissolved in 0.1 mol l^{-1} NaOH, and diluted 10× with solvent A [0.1 mol l^{-1} $(NH_4)H_2PO_4$, pH 3.5, and methanol (55:45, v/v)], and applied to the column. Solvent B was methanol. The chromatography was performed using a 40 min gradient at a flow rate of 0.5 ml min^{-1}, increasing the proportion of solvent B from 60% to 75%. Solvent B concentration was then raised to 100% and maintained for a further 30 min.

Mass spectrometry

Mass spectra were acquired using a Finnigan LCQ-Duo ion trap mass spectrometer (Finnigan, ThermoQuest Inc., San Jose, CA, USA). Samples from HPLC fractionation of hemosomes, obtained as described above, were diluted 10× with HPLC-grade methanol (Carlo Erba, Milan, Italy), and introduced into the electrospray source by injection of 10 μl samples through a 50 μm fused silica capillary at a flow rate of 5–10 μl min^{-1}. Spectra were acquired at 3 s per scan over a 200–2000 mass/charge (*m/z*) range. Source voltage was 4.5 kV, and the electrospray ionisation (ESI) capillary voltage was set at 31.5 V. Vaporizer and capillary temperatures were set at 32 and 200°C, respectively. Spectra were collected in positive ion mode after instrument mass calibration with an authentic haem standard (Sigma, St Louis, MI, USA).

FTIR spectrometry

Dried samples of standard haemin (Sigma) or hemosome core (isolated as described above) were used to obtain reflectance spectra, acquired for 60 cycles with a Fourier Transform Infrared (FTIR) spectrometer (Nicolet, Magna 550).

Elemental mapping

To evaluate iron and nitrogen concentrations in different

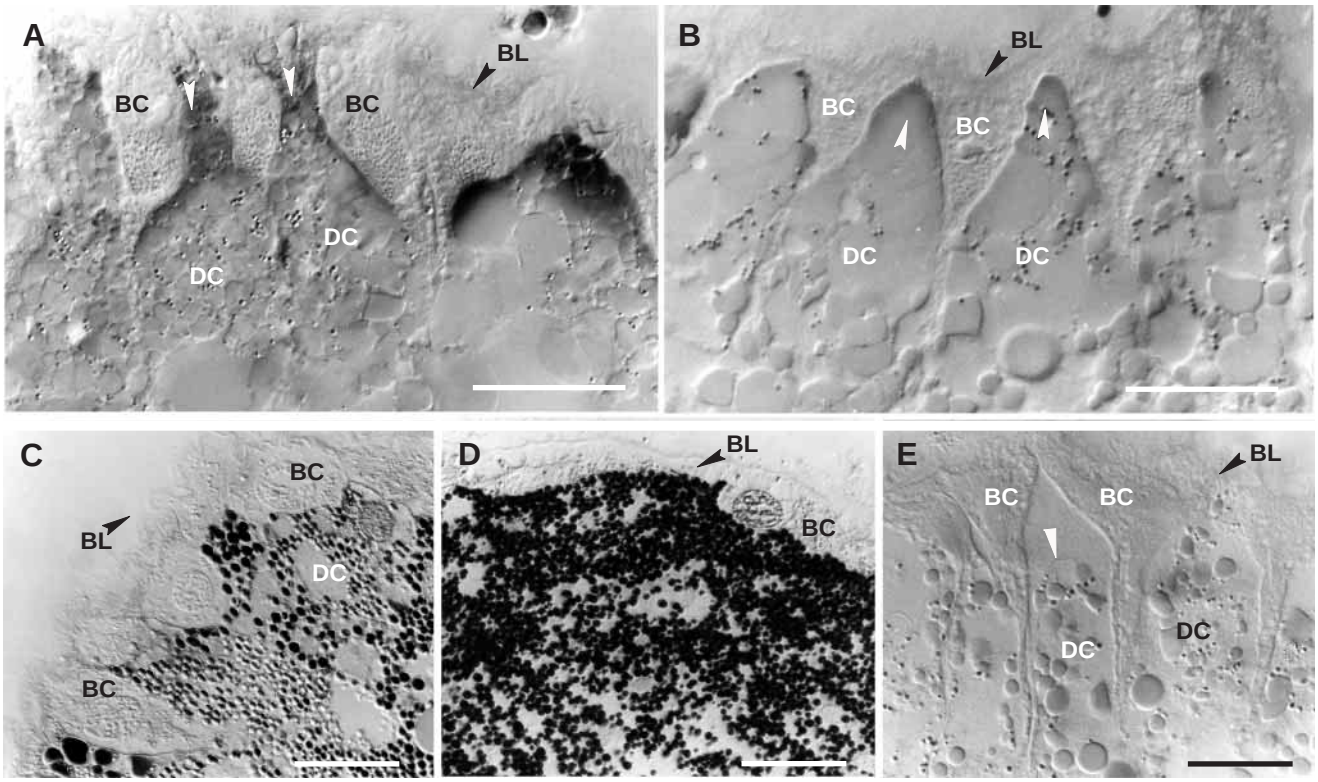

Fig. 1. Haem peroxidase activity in the tick midgut epithelium during blood digestion. Midgut sections were stained for 3-3-diaminobenzidine (DAB) oxidation, and observed by differential interference contrast (DIC) microscopy. (A) First day after blood meal (ABM); arrowheads show contact between digest cells (DC) and the basement membrane (BL). Scale bar, 35 μm. (B) Third day ABM; arrowheads show the diminished contact between digest cells and basement membrane. Scale bar, 35 μm. (C) Day 15 ABM. Scale bar, 60 μm. (D) Day 20 ABM. Scale bar, 60 μm. (E) Day 5 ABM, negative control without H_2O_2; arrowheads show detachment of digest cells. Scale bar, 40 μm. BC, basophilic cell.

compartments of the digest cell, samples were processed as described for transmission electron microscopy, without post-fixation, and analysed using a Zeiss 912 Omega transmission electron microscope with a LaB_6 filament. For elemental mapping, unstained ultra-thin sections (nominal thickness 30–50 nm) were analysed at 120 kV with an objective aperture of 12.5 mrad and an energy-selecting aperture of 20 eV. Images were digitised with a 14-bit slow scan CCD camera (Proscan, Germany) controlled by an image analysis system (ESI Pro, SIS GmbH, Germany). Elemental maps were calculated with the three-window power-law method for iron L edge (pre-edge images at 660 and 690 eV, post-edge at 720 eV) and the two-window method was used for nitrogen K edge (pre-edge images at 390 eV, post-edge image at 410 eV).

Results

In order to follow the fate of haem after the final large blood meal, the midgut of *Boophilus microplus* was stained for haem-peroxidase activity against DAB and observed by means of DIC microscopy (Fig. 1). On the first day after dropping, the epithelium showed two distinct cellular types bound to the basal membrane, that we named basophilic cells and digest cells, in agreement with previous descriptions (Agbede and Kemp, 1985) (Fig. 1A,B). Basophilic cells have been categorised by some authors as stem cells that generate digest cells (Agyei and Runham, 1995), whereas others describe basophilic cells as a differentiated cell lineage (Agbede and Kemp, 1985). The basophilic cells were caliciform in shape on the first and third days, but had changed to a round shape by day 15 (Fig. 1C) and were finally flattened by day 20 (Fig. 1D).

On days 1–3 after the blood meal (ABM), digest cells are attached to the gut wall and have areas of direct contact with the basal lamina, which are reduced by day 5 and are no longer observed by the days 15 and 20 (Fig. 1). On the first day, the haem-peroxidase label was strongly concentrated on the basal surface of the digest cells, near to the basal lamina, suggesting that these cells were delivering haem to the haemolymph (Fig. 1A). By the third day the digest cells began to show heavily stained dark granules (Fig. 1B), hereafter called hemosomes, which progressively increased both in size and number and eventually occupied most of the cell cytoplasm 2 weeks ABM (Fig. 1D,E). The development of hemosomes occurred in parallel to the progressive detachment of the digest cells from the gut wall, which would presumably result in reduction of metabolite transfer to the haemocoel. These results imply a role for hemosomes as a site of haem sequestration.

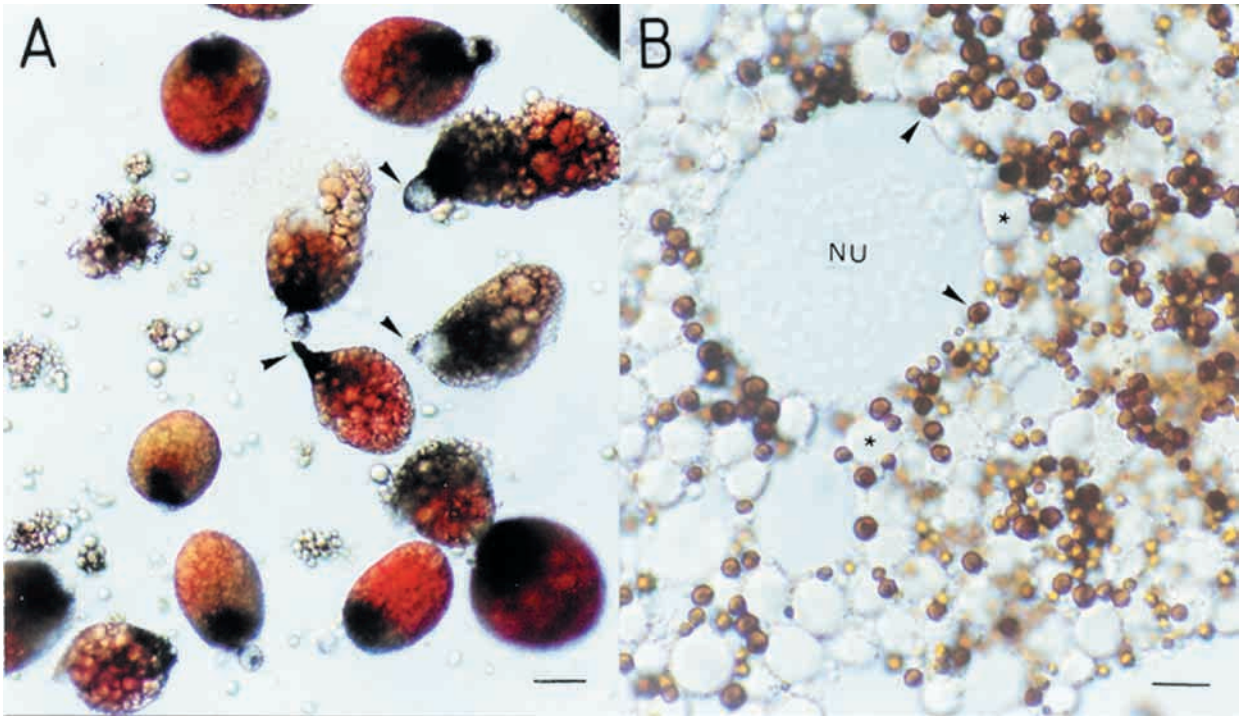

Fig. 2. Digest cells on the third day ABM, isolated and observed by differential interference contrast (DIC) microscopy. (A) Digest cells separated from the midgut were observed without a coverslip. Arrowheads show the site of attachment to the gut epithelium. Scale bar, 55 μm. (B) Digest cell observed with a coverslip, at higher magnification. Hemosomes are concentrated in the perinuclear area (arrowheads). Asterisks indicate digestive vesicles. Scale bar, 5 μm. NU, nucleus.

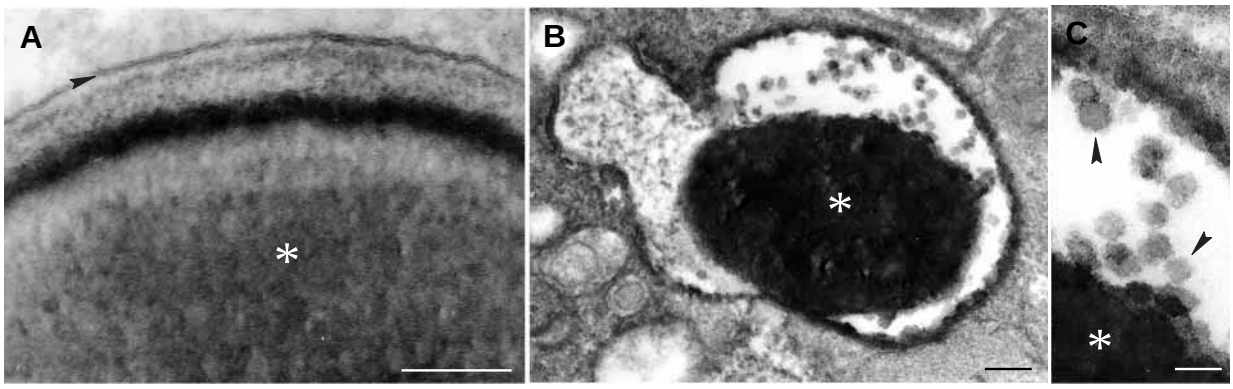

Fig. 3. Hemosome ultrastructure and formation. Transmission electron microscopy of the cytoplasm region of digest cells. (A) Mature hemosome. The arrowhead indicates a lipid bilayer membrane. Scale bar, 150 nm. (B) Growing hemosome. Scale bar, 150 nm. (C) Detail of B. The hemosome is formed by association of homogeneous sub-particles (arrowheads), possibly migrating towards the core (asterisk). Scale bar, 50 nm.

Digest cells from the third day ABM were isolated by opening the midgut and gently washing the luminal content with phosphate-buffered saline. Observation of intact cells by light microscopy (Fig. 2A) revealed that blood digestion is clearly polarized in these large (>90 μm) cells. Typically, one side of their cytoplasm is occupied by large digestive vacuoles displaying an intense red colour, suggesting the presence of undigested haemoglobin, whereas hemosomes are concentrated on the other side of the cell (Fig. 2A). When squeezed between the lamina and the coverslip, the perinuclear location of the hemosomes in the digest cells is clearly shown during this initial phase of blood digestion (Fig. 2B).

When the midgut was stained with DAB and observed by transmission electron microscopy, the fully grown, mature hemosomes appear as strongly labelled, very electron-dense structures (Fig. 3A). Hemosomes are delimited by a membrane and have a very compact core, which is surrounded by a cortical region encompassing several distinct layers. Fig. 3B shows a growing hemosome not yet fully developed, displaying strong peroxidase activity near the surface of the

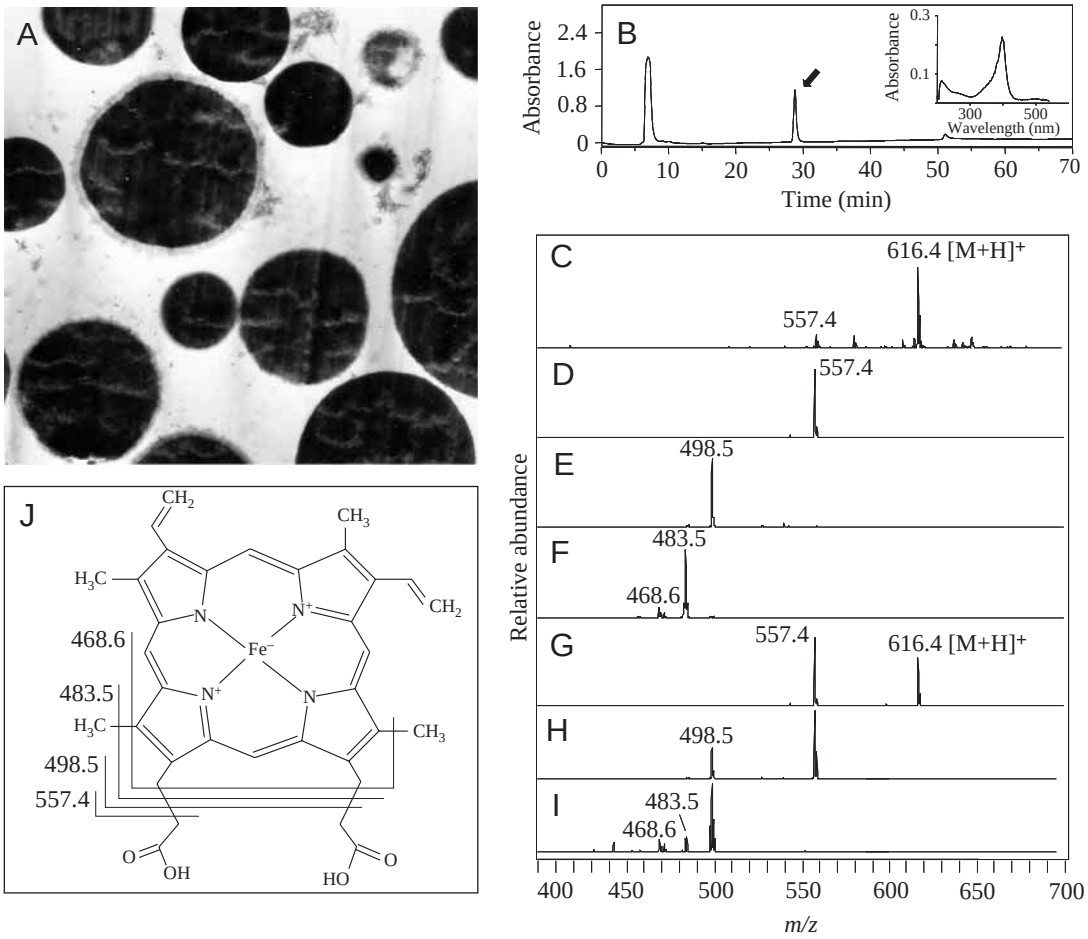

Fig. 4. Hemosome composition as determined by high performance liquid chromatography (HPLC) and electrospray-ionisation mass spectrometry (ESI-MS). (A) Transmission electron microscopy of hemosome cores after isolation through a Percoll gradient and washing with deionised water. Scale bar, 1.0 μm. (B) Reverse-phase HPLC fractionation of the hemosome content, monitored at 220 nm. Arrow, a peak with a retention time similar to a haem standard; inset, the light absorption spectrum of the same region. (C–F) Positive-ion mode electrospray-ionisation mass spectrometry (ESI-MS) profile of the intact haem fraction (C) and tandem ESI-MS (ESI-MS/MS) spectra of its daughter ions (D–F). (G–I) ESI-MS/MS spectra of standard haem. (D,G) Daughter ions of *m/z* 616.4. (E,H) ESI-MS/MS spectra of the daughter ion *m/z* 557.4. (F,I) Tandem ESI-MS spectra of the daughter ion *m/z* 498.5. (J) Structure of the iron-protoporphyrin IX and the proposed assignment for its observed daughter ions.

vesicle. The core of the hemosome comprises an assembly of homogeneous particles of haem aggregates (mean size 40±3 nm), which seem to detach from the hemosome membrane, suggesting a model mechanism for the formation of this structure (Fig. 3C).

Hemosomes isolated by Percoll gradient ultracentrifugation were suspended in deionised water, frozen, thawed and centrifuged again. The pellet obtained, when observed by transmission electron microscopy, shows the core of the hemosome displaying almost its original appearance, only the more external layers being removed by this treatment (Fig. 4A). The hemosome pellet was dried, weighed and the haem content measured by the alkaline pyridine derivative, revealing that haem alone accounts for 90±3% of the composition of this organelle. The haem aggregate from the hemosome preparation was dissolved in NaOH, isolated by reverse phase chromatography and analysed by electrospray ionisation mass-spectrometry (ESI-MS) (Fig. 4B–I). A major singly charged ($[M+H]^+$) ion species was observed at *m/z* 616.4 (Fig. 4C). When submitted to sequential fragmentation by tandem ESI-MS/MS, this ion species gave rise to daughter ions of *m/z* 557.4, 498.5, 483.5 and 468.6 (Fig. 4D–F). The same ion fragments were obtained from an authentic haem standard (Fig. 4G–I). Therefore, we can conclude that hemosome-derived fractions showing absorption at the Soret band (398 nm) are actually iron-protoporphyrin IX (Fig. 4J). Furthermore, ESI-MS data show that there is no covalent modification of the haem molecule upon haemoglobin digestion and accumulation into the organelle. However, the FTIR spectrum of hemosome is clearly distinct from that of standard haem (Fig. 5). Some peaks found in the hemosome core could be assigned to other non-haem components of the aggregate (indicated by asterisks in the figure). A remarkable feature, however, is the absence of the characteristic 1704 cm^{-1}

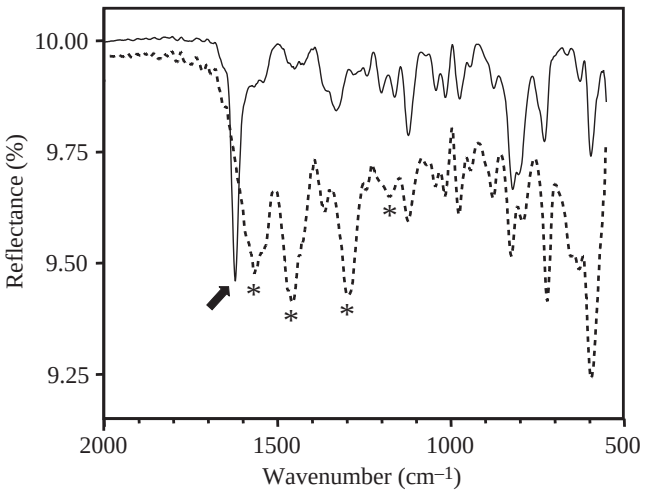

Fig. 5. Fourier Transform infrared (FTIR) spectrum of the hemosome. Hemosomes were isolated as described in Materials and methods. Shown are FTIR spectra of the hemosome core (broken line) and haem standard (solid line). Arrow, haem carboxylate peak at 1704 cm^{-1}. Asterisks, peaks at 1652 cm^{-1}, 1546 cm^{-1}, 1392 cm^{-1} and 1279 cm^{-1} that can be designated as non-haem components of hemosome.

carboxylate stretch (arrow), which suggests that these groups are interacting with other components of the aggregate.

Digestive vacuoles are protein-rich structures, and therefore a high local concentration of nitrogen was expected. Haem accumulation, on the other hand, should result in a local increase of iron concentrations. Elemental mapping of the hemosome by energy-filtering transmission electron microscopy was used to evaluate nitrogen and iron concentrations. Based on the results shown in Fig. 6, we propose a maturation cycle for this organelle. Typically, hemosomes are smaller on day 4 ABM and present the iron-rich core surrounded by a boundary, with lower density of both iron and nitrogen. They were frequently found in close association with the much larger digestive vacuoles, which showed a strong nitrogen signal, together with a lower concentration of iron. On day 8 ABM, hemosomes larger in size and of compact aspect were more frequent and the clear, low-iron boundary between the core and the surface was no longer observed in these vesicles. By day 20 ABM, no more digestive vacuoles were found and the hemosomes had become less dense structures, with several distinct internal layers on both the iron and nitrogen maps.

Discussion

In contrast to most nucleated animal cells that synthesise their own haem, the cattle tick *Boophilus microplus* is not capable of synthesising this molecule, and therefore is strictly dependent on the vertebrate haemoglobin (Braz et al., 1999). This deficiency should be counterbalanced by the capacity to acquire haem from ingested vertebrate blood. On the other hand, haem is a potentially toxic molecule, capable of promoting oxidation of lipids, proteins and DNA (Ryter and Tyrrell, 2000). Here we show that haem can either be absorbed through the gut or detoxified by accumulation into specialised organelles, herein called hemosomes.

We have shown previously that during the initial days after a blood meal (ABM) there is a huge accumulation of haem in the oocytes (Braz et al., 1999), and also have presented evidence that this haem originates from the haemolymph and is delivered by a haemolipoprotein called HeLp (Maya-Monteiro et al., 2000). These results are in agreement with the observed intense concentration of haem at the basal surface of the digest cell during the initial days ABM, which coincide with the period of most intense vitellogenic oocyte growth, suggesting that by this time the absorption pathway seems to predominate over hemosome formation (Fig. 1A,B). This phenomenon is dependent on the accumulation of vitellin, the major yolk protein of oviparous animals (Sappington and Raikhel, 1998), which in ticks is a haemoprotein (Rosell and Coons, 1991) that functions as a haem reservoir to support embryo development (Logullo et al., 2002).

One important question concerns the cytoplasmic route taken by haem on its way from the digestive vesicles to the haemocoel. The haem might be exocytated by the digest cells at its basal surface or, alternatively, might be transported through the membrane of the digestive vesicles to the cytoplasm and then transferred to the haemocoel *via* the cellular membrane. Transmission electron microscopy, using 3-3-diaminobenzidine, did not provide a definitive answer to this question, but, based on haem-peroxidase activity, we observed that it is not only the digestive vesicles that are stained by DAB, but also the cytoplasm itself, which seems to have a higher haem content compared to the cytoplasm of the neighboring basophilic cells (F. A. Lara, data not shown). We were not, however, able to find DAB-positive exocytic vesicles at the basal surface of digest cells. Taken together these data would favor the hypothesis of a haem transport pathway from the digestive vesicles through the cytosol to reach the haemocoel at the basal surface.

After the first week ABM, the intensity of haem absorption seems to decrease, with a decline in the concentration of haem close to the haemocoel and reduction of contact of digest cells with the basal lamina (Fig. 1D,E), when compared to the previous days. At this time, haem released from haemoglobin in midgut cells becomes directed to hemosomes, which are the most prominent organelle of senescent digest cells (Fig. 1D,E). Almost 10% of the haem present in the meal of an adult fully engorged female is found in its ovary (O'Hagan, 1974) and by the end of digestion (2 weeks ABM) >98% of the haem in the gut is associated with hemosomes (F. A. Lara, data not shown). These structures have been described by other authors as residual bodies, and are usually excreted along with the faeces at the end of digestion (Tarnowski and Coons, 1989). Iron was suggested to be their main constituent and therefore these structures were named siderosomes in earlier literature (Walker and Fletcher, 1987).

Here we show that haem, and not iron, is the major

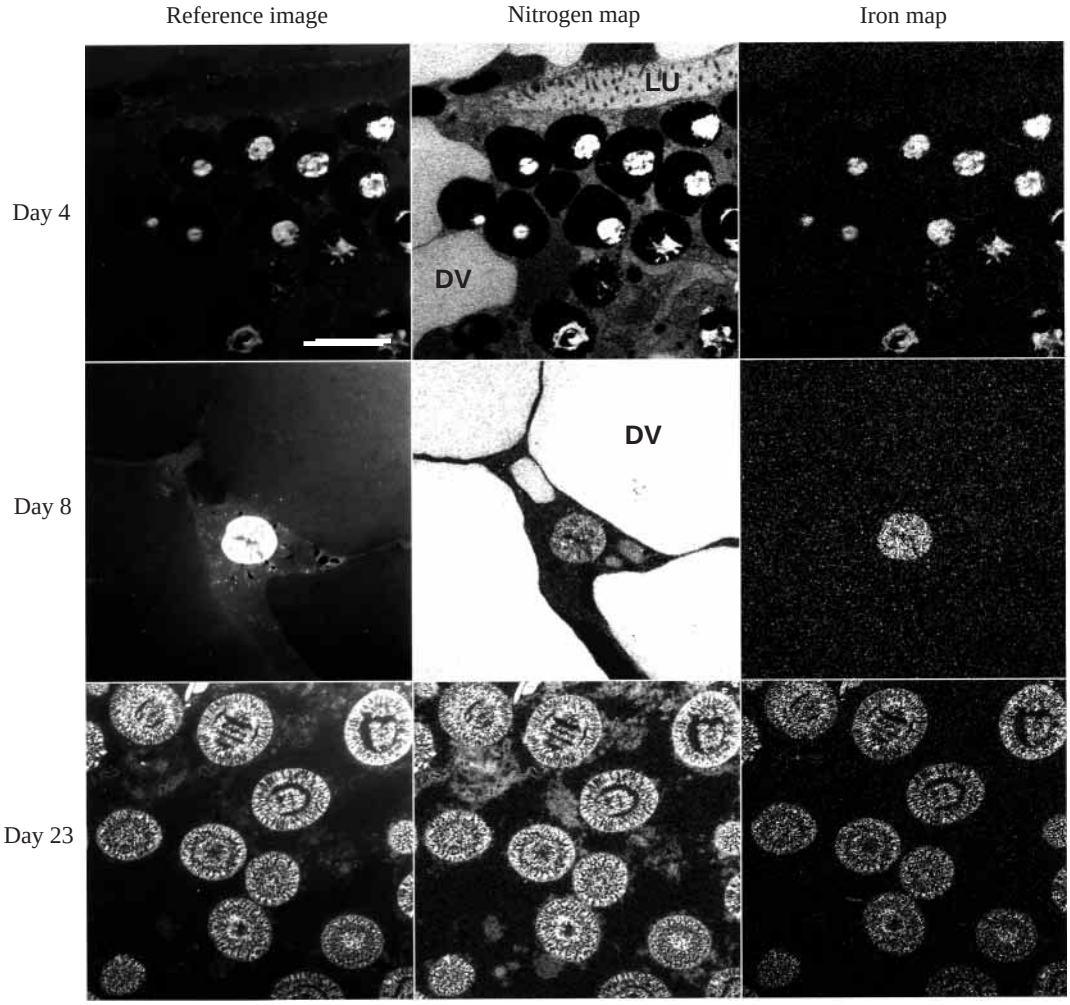

Fig. 6. Elemental mapping of hemosome development. Cytoplasm of digest cells was observed by energy-filtering transmission electron microscopy. Each row of panels shows a section from cells on different days ABM (4, 8 and 20 ABM, from top to bottom). (Left) A reference image obtained at 250 eV, which depicts a structure-sensitive contrast. Nitrogen (middle) and iron (right) distributions are shown. DV, digestive vesicles; LU, lumen. Scale bar, 5 μm.

component of these vesicles, accounting for 90% of its mass. By means of sequestering most of the haem in excess of its physiological demand inside the hemosome, the tick counteracts any haem toxicity that could otherwise result in severe tissue damage. Thus hemosome formation should be regarded as a detoxification mechanism, functionally equivalent to the formation of haemozoin, a crystalline haem aggregate that occurs in other haemoglobin-eating parasites such as *Plasmodium* (Slater et al., 1991), *Rhodnius prolixus* (Oliveira et al., 1999) and *Schistosoma mansoni* (Oliveira et al., 2000). The haem aggregate found in the tick hemosome is not haemozoin, however, as revealed by its distinct FTIR spectra (Fig. 5) and by the absence of birefringence observed by polarizing microscopy and the lack of X-ray diffraction (M. F. Oliveira, data not shown).

With respect to hemosome structure, the absence of a 1704 cm^{-1} carboxylate stretch in the FTIR spectrum (Fig. 5) suggests that haem association to non-haem components of the hemosome involves the lateral propionate chains of the porphyrin ring. Haem iron-carboxylate bonding – as found in haemozoin – can be excluded by the absence of the 1660 cm^{-1} peak, suggesting that the hemosome core comprises a new type of haem-based supramolecular structure. Characterization of the non-haem components of the hemosome and of the molecular structure of this haem aggregate are underway in our laboratory. Another essential feature is the presence of a delimiting membrane (Fig. 4), indicating that the hemosome and digestive vacuoles are distinct structures and pointing to the existence of a haem transport system between both cellular compartments. Blood-feeding organisms are found in very different taxonomic groups and all have haem as the main end product of haemoglobin digestion, frequently being described under the generic denomination of haematin in the previous literature. Structural characterization of a haem aggregate is only available, however, for haemozoin. The presence of haemozoin has been investigated in several species of haematophagous animals but with negative results in most cases, except for *Plasmodium*, *Rhodnius* and *Schistosoma*, as

mentioned above (Oliveira et al., 1999, 2000). To our knowledge this is the first characterization of a non-haemozoin haem aggregate. A goal for future research is to compare the structural organization of haem aggregates in different blood-feeding organisms.

In most organisms, during turnover of haem proteins the protoporphyrin ring is cleaved by haem oxygenase and the iron atom is eventually reused for *de novo* synthesis of haem (White and Granick, 1963; Ponka, 1997). The only exceptions to this are some pathogenic protozoa and bacteria, in which a complex array of proteins has evolved to take up haem efficiently from the vertebrate host (Wandersman and Stojiljkovic, 2000). Among higher organisms, the tick *Boophilus microplus* (Braz et al., 1999) and the hemipteran *Rhodnius prolixus* (Braz et al., 2002), both blood-sucking arthropods, are the only exceptions reported to date. We have recently shown that HeLp (Haem Lipoprotein), the major lipoprotein of *Boophilus microplus* haemolymph, is capable of binding haem and delivering it to the tick tissues (Maya-Monteiro et al., 2000). The same protein has also been described in two other tick species and therefore its presence may be a common feature of ticks (Gudderra et al., 2002). During the first week ABM, haem absorption is more evident and digest cells that attach to the basal lamina may have direct contact with the haemocoel, so it is conceivable that direct transfer of haem from digest cells to HeLp may occur. Haem is never found free in its unbound form, however, due to its potential toxic effects previously mentioned, and is always associated to proteins. The transfer of haem to HeLp must therefore be preceded by transfer from the digestive vacuole to the surface of the digest cell. The precise pathway may include the hemosomes, which also concentrate close to the basal lamina at the same time, as an intermediary compartment. Alternatively, the hemosome may represent a divergent route taken by haem when its levels exceed the tick's needs. Haem-binding proteins participating in intracellular trafficking may be the targets for the development of new methods of acari control, as they participate in a detoxification pathway that is specific for this group of animals.

Taken all together, our data describe a new process for haem detoxification by means of sequestration inside the hemosome. The ability to form this structure is proposed to be an important adaptation of this organism in order to use vertebrate blood as its sole food source.

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