Short communication

Haemozoin in *Schistosoma mansoni*

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Blood feeding is a feature that is shared by a large variety of organisms from Protozoa to mammals, and regardless of their diverse phylogenetic origins most of them digest haemoglobin. This process results in the release of peptides, amino acids and the prosthetic group haem. Free haem is very toxic and several mechanisms have been developed in order to protect organisms against its deleterious effects [1]. In *Plasmodium* parasites, most of the free haem is detoxified by sequestration into a dark-brown crystalline material known as malaria pigment or haemozoin (Hz) [2,3]. Until recently, this pigment had only been found in Plasmodia. However, we have identified Hz in the blood-sucking insect *Rhodnius prolixus* [4,5].

Schistosoma mansoni, the main aetiological agent of human schistosomiasis, digests large amounts of haemoglobin in the host circulation in order to complete development and sexual maturation [6,7]. During this process, haem derived from haemoglobin breakdown is released inside the worm gut. It has been proposed that haem is the end product of haemoglobin digestion and that the worms continuously regurgitate it as a dark-brown material called *Schistosoma* pigment

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(SP) [8,9]. Previous works showed that malaria and *Schistosoma* pigments could not be distinguished in pathological examination or by histochemical, spectroscopic and microscopic analysis [9,10]. Moreover, both pigments accumulate and may remain for many years in human livers [9,11]. However, further comparison of *Plasmodium* Hz and SP by electron microscopy led to the conclusion that the structures of these pigments were in fact distinct [12,13]. Since then, no further studies were carried out in order to elucidate the exact structure of SP, which has been widely referred to as haematin, the monomeric hydroxy-liganded form of haem. This controversy, and the importance of haem aggregation in the physiology of *Plasmodium* and *R. prolixus*, led us to further explore the nature of the pigment produced upon haemoglobin breakdown in *S. mansoni*.

Using methods previously described for malarial Hz extraction [14], we obtained a dark-brown insoluble pellet from homogenized adult females of *S. mansoni*. Observation of this pellet using polarized light microscopy revealed numerous birefringent granules indicating that SP, like Hz, has a crystalline structure (data not shown) [15]. SP can be readily solubilized in 0.1 M NaOH, a condition in which the iron-carboxylate bonds of Hz are broken [2,16], giving rise to an absorption spectrum identical to that of monomeric haem (data not shown). Intact SP resuspended in 0.1 M NaHCO$_3$, pH 9.1, showed an absorption spectrum markedly distinct from the absorption of monomeric haem and very similar to that of *R. prolixus* Hz, with broad absorbance peaks near 450 and 660 nm (Fig. 1A). Fourier transform infrared spectroscopy (FTIR) of SP in KBr pellets revealed a pattern identical to Hz from *Plasmodium* [2] and *R. prolixus* [4,5], with specific absorption peaks at 1210 and 1663 cm^{-1} which are derived from iron-carboxylate bonds responsible for Hz structure (Fig. 1B). Haem-acetate adducts exhibit a similar FTIR spectrum, but these adducts are freely soluble under conditions where Hz is not [17]. In order to investigate this issue, we compared the solubilities of haem and SP. Haem, in its free form, is readily soluble in sodium bicarbonate buffer, pH 9.1, whereas SP is virtually insoluble (data not shown). Taken to-

gether, these results unequivocally identify the pigment isolated from *S. mansoni* as Hz. Attempts to identify the factors capable of inducing haem aggregation in *S. mansoni* are currently under way in our laboratory.

Adult schistosomes exhibit remarkable sexual dimorphism. Moreover, there is a striking difference in color between males and females, the females displaying an intensely dark-brown pigmented body and males being light brown. Females have significantly higher rates of haemoglobin ingestion and breakdown than males [6], which probably leads to differential ability to produce and accumulate SP. This prompted us to compare the contents of total haem and Hz in adult males and females of *S. mansoni*. Fig. 2 shows that total haem and Hz contents are indeed much higher in females than in males. Further-
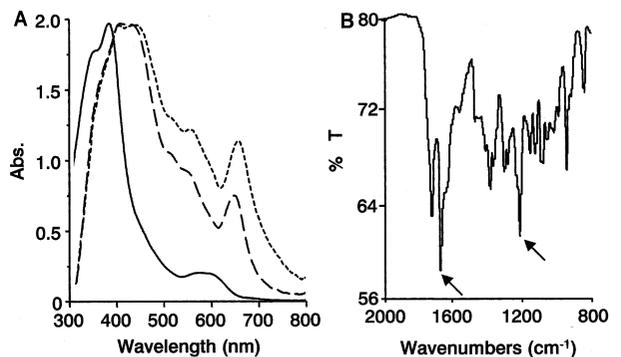

Fig. 1. Characterization of Hz from *S. mansoni*. *S. mansoni* strain LE was maintained in *Biomphalaria glabrata* snails and in Syrian hamsters. Adult worms were obtained by mesenteric perfusion of hamsters, 42 days after infection, as previously described [29]. Hz was extracted from *S. mansoni* based on methods previously described for malarial haemozoin [14]. Adult female worms homogenized in 0.15 M NaCl at 25°C were centrifuged at $1000 \times g$ for 10 s. The pellet was discarded and the suspension was re-centrifuged at $20\,000 \times g$ for 20 min. The dark-brown pellet was washed four times with 0.1 M NaHCO$_3$ buffer + 2.5% SDS, pH 9.1. (A) Normalized absorption spectra of haemin (solid line), *Rhodnius* Hz (dashed line) or SP (dotted line) in 0.1 M NaHCO$_3$, pH 9.1 using a GBC/UV-920 (GBC) spectrophotometer. (B) FTIR spectrum (KBr pellets) of the final material obtained from adult female homogenates subjected to Hz extraction. The arrows indicate the characteristic Hz absorbance peaks at 1210 and 1663 cm^{-1}. FTIR spectroscopy was carried out in KBr pellets prepared from dried samples and spectra were acquired for 32 cycles in a FTIR spectrometer (Nicolet, model Magna 550).
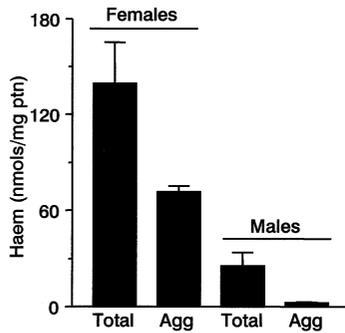
Fig. 2. Conversion of haem into Hz in *S. mansoni* males and females. Forty-two-day-old adult worms obtained by perfusion of Syrian hamsters were homogenized in 1 ml of 0.15 M NaCl containing 0.5 mM benzamidine, 50 μg ml^{-1} soybean trypsin inhibitor, 0.02 mg ml^{-1} antipain, 10 μM pepstatin, 0.1 mM Zn acetate and 2 mM dithiothreitol. Protein content in homogenates was measured using bovine serum albumin as standard [30]. After that, samples were treated with 1 mg ml^{-1} of proteinase K in PBS, pH 7.4, at 37°C for 12 h. For total haem determination, female and male homogenates were incubated with 0.1 M NaOH under continuous shaking for 30 min and centrifuged at 20 000 × *g* for 10 min and the supernatant used for total haem measurements (Total). Alternatively, homogenates were subjected to the Hz extraction protocol for determination of aggregated haem (Agg). In both cases, haem quantification was spectrophotometrically determined at 400 nm in an Ultrospect U2000 spectrophotometer (Pharmacia). Bars represent means ± S.E. from four different experiments.

more, females are more efficient than males in converting haem into Hz: females detoxify more than 50% of the total haem into Hz, while males convert only about 10%. These results are in line with previous reports indicating that females have an intense metabolism related to the nutritional requirements to support oogenesis and hence are more efficiently adapted to blood digestion than males [18]. The differential capacity of Hz synthesis between the sexes in *S. mansoni* could be due to various factors. First, females ingest and digest more erythrocytes, and therefore have higher levels of haem inside their guts than males. Furthermore, it is conceivable that females have a higher haem aggregation activity than males, which accounts for a higher amount of pigment found in females. Alternatively, males could rely on other mechanisms of haem detoxification, such as degradation by haem oxygenase [19] or mediated by reduced glutathione [20], which could account

for the reduced conversion of haem into Hz compared to females. Whatever the case, there is a clear difference between males and females in the way they deal with haemoglobin-derived haem. Moreover, females are dependent on pairing with males in order to complete sexual maturation [21], gene expression [22], red blood cells ingestion [23] and possibly Hz synthesis. In fact, previous works showed that unisexual infection with females of *S. mansoni* produces stunt worms, which are sexually immature and have significantly less pigment than mated females [23].

The process of Hz crystallization has been known for quite some time in the protozoan *Plasmodium* [2], was very recently identified by us in the insect *R. prolixus* [4,5] and is now shown to occur in the helminth *S. mansoni*. Therefore, similar to *Plasmodium* and *R. prolixus*, *S. mansoni* produces Hz in order to detoxify the haem derived from haemoglobin digestion. Indeed, Hz synthesis in *S. mansoni* seems to occur early in development since very small pigment granules were detected inside schistosomula and lung worms guts when observed under light microscopy (data not shown). The finding of haem aggregation into Hz in these widely different organisms suggests that other haematophagous animals may solve the problem of ingestion of large amounts of potentially toxic haem by following the same strategy. In this regard, it is interesting to note that previous reports have indicated the presence of pigments derived from haemoglobin digestion in *Diclidophora merlangi* and *Fasciola hepatica* [24]. Nevertheless, preliminary results from our group indicate that other haematophagous animals such as mosquitoes (*Aedes* and *Anopheles*) and the hard tick (*Boophilus microplus*) do not produce Hz (data not shown). It thus appears that the capacity to synthesize Hz may not be a universal solution employed by all haematophagous organisms, but rather may be associated with the chemical environment of the digestive process: *Plasmodium*, *Rhodnius* and *Schistosoma* digest haemoglobin in an acid environment, and it is noteworthy that the process of haem aggregation is strongly pH-dependent [25]. Aggregation requires that half of the haem propionate chains be dissociated, which occurs at pH values near the pK_a of haem (around pH 4.8) [26].

In conclusion, the results presented here indicate that haem detoxification into Hz may be an important feature in the adaptation of different organisms to haematophagy. Moreover, in the specific case of *S. mansoni*, Hz synthesis could be an interesting target for chemotherapy through the use of compounds that could block the process of haem aggregation in vivo, leading to a potentially toxic situation of haem overload in the parasites. This approach could become all the more important in view of the well-reported cases of increased resistance of *S. mansoni* to commonly used antihelminthic drugs such as hycanthone, oxamniquine and praziquantel [27,28].

Acknowledgements

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