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Recent advances in understanding the mechanism of hemozoin (malaria pigment) formation

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Abstract

The recent literature on hemozoin/β-hematin formation is reviewed, with an emphasis on the mechanism of its formation. Recent findings from unrelated organisms that produce hemozoin, namely the malaria parasite *Plasmodium falciparum*, the worm *Schistosoma mansoni* and the kissing bug *Rhodnius prolixus* all of which consume human hemoglobin show that the formation of this crystalline substance occurs within or at the surface of lipids. Biomimetic experimental models of the lipid–water interface as well as computational studies indicate that these lipid environments are probably extraordinarily efficient at producing hemozoin. A rethink is now needed, with a new emphasis on Fe(III)PPIX in non-aqueous environments that mimic lipids and indeed within the lipid environment itself. These findings are explored and discussed in the context of earlier studies on β-hematin formation.

Keywords: Hemozoin; β-Hematin; Malaria pigment; Schistosoma pigment

1. Introduction

When examined under a microscope, a striking feature of the trophozoite stage of a malaria parasite is the presence of a dark brown-black substance known as malaria pigment or hemozoin. This substance is found within a lysosome-like compartment known as a food vacuole (or digestive vacuole). The pigment is released into the blood of the host each time the parasite completes a blood cycle and eventually deposits in internal organs, causing noticeable discoloration. This phenomenon was first reported by Giovanni Maria Lancisi in a book published in 1717 [1], predating the discovery of the malaria parasite by more than 150 years. Later hemozoin played an important part in elucidating the role of mosquitoes as vectors of the parasite, acting as a visible tracer [2]. In these early studies, the pigment was assumed to be melanin, but in 1911 Wade H. Brown demonstrated that the chromophore is heme [3]. It was to be almost 80 years before Fitch and Kanjananggulpan demonstrated that hemozoin consists solely of ferriprotoporphyrin IX (Fe(III)PPIX) [4], probably identical to β-hematin, an insoluble Fe(III)PPIX precipitate first described in the 1930s [5]. Subsequently Slater et al. demonstrated by X-ray diffraction, infrared spectroscopy and solubilization studies that hemozoin is indeed identical to β-hematin and showed by EXAFS spectroscopy that β-hematin contains bonds between the propionate group of one iron porphyrin and the Fe(III) center of its neighbor [6]. Any lingering doubts that hemozoin and β-hematin are identical were removed in 1997 when Bohle et al. demonstrated by high resolution synchrotron X-ray diffraction that lyophilized parasitized erythrocytes give an identical diffraction pattern to β-hematin [7]. Finally, in 2000 Pagola et al. determined the structure of β-hematin from the X-ray powder diffraction pattern (Fig. 1) [8].

Hemozoin is produced as an end product of heme released during the digestion of host hemoglobin by the malaria parasite and is believed to be a detoxification pathway in the parasite. Fe(III)PPIX produced by autoxidation

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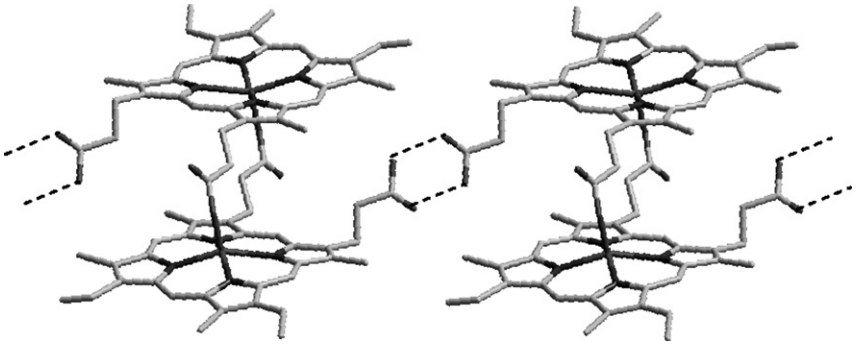

Fig. 1. The structure of hemozoin/β-hematin. Dotted lines represent hydrogen bonds.

of heme released from hemoglobin is known to be capable of causing lipid peroxidation [9] and to destabilize membranes through a colloid osmotic mechanism [10]. Packaging Fe(III)PPIX into compact and highly insoluble hemozoin crystals decreases its pro-oxidant capacity [11] and likely also avoids colloid osmotic effects. If the parasite were to degrade Fe(III)PPIX, as mammals do using the enzyme heme oxygenase, it would be faced with sequestering the vast quantity of iron released since free Fe(III) is also highly toxic. It is handled by specialized transport and binding proteins in higher organisms [12].

Nonetheless, there has been uncertainty about whether hemozoin formation is the main fate of heme in the parasite *Plasmodium falciparum* [13,14]. In two studies a large discrepancy was found between the total iron content of parasitized erythrocytes on the one hand and the sum of undigested hemoglobin and hemozoin on the other. Zhang et al. claimed that about 70% of the Fe(III)PPIX is degraded in a glutathione dependent process outside of the parasite food vacuole where hemozoin is found [13]. Loria et al. claimed a similar degree of Fe(III)PPIX degradation occurs within the food vacuole [14]. In both of these studies hemozoin was quantified by dissolution in 0.1 M NaOH and measurement of the Soret band of Fe(III)PPIX in basic aqueous medium. However, when in a later study the iron content of *P. falciparum* parasitized erythrocytes, isolated parasites, food vacuoles and hemozoin were each separately measured colorimetrically by release of total iron followed by coordination of Fe(III) with ferrozine to form a colored complex, it was found that 70–100% (95% CI) of the parasite iron is found in the hemozoin [15]. This conclusion was unequivocally supported by Mössbauer spectra of freeze-dried intact parasites in which the only detectible iron signal was shown to be that of hemozoin and the magnitude of background scatter limits other iron species to no more than 5% of the total iron content [15]. Elemental mapping of transmission electron micrographs by electron energy loss spectroscopy further supported the conclusion that hemozoin formation is the overwhelming fate of heme released in the parasite [15]. Collectively, these techniques demonstrate that at least 95% of the heme released in the parasite is converted to hemozoin. Recently Gligorijevic et al. have studied hemozoin formation in single live intra-erythrocytic parasites using spinning disk confocal microscopy [16]. The quantity of hemozoin determined in this work from the volume of crystals observed (15 μmol/10^{10} cells) corresponds to 88% of the heme present in the erythrocyte (17 μmol/10^{10} cells), confirming that heme must be almost entirely converted to hemozoin.

Over the last decade, hemozoin has been discovered in a number of other blood-feeding organisms, including the insect *Rhodnius prolixus* (the kissing bug) [17], helminth worms *Schistosoma mansoni* (the causative agent of schistosomiasis or bilharzia) [18,19] and *Echinostoma trivolvis* [20] and the bird-infecting protozoan *Haemoproteus columbae* [19]. These discoveries have both broadened the interest in hemozoin and aided in understanding the mechanism of hemozoin formation.

Apart from being interesting in its own right, hemozoin formation is also important as a target of antimalarials such as chloroquine. In 1992, Slater and Cerami reported that 4-aminoquinoline and quinoline methanol antimalarials inhibit parasite extract induced β-hematin formation and suggested that they inhibit an enzyme responsible for hemozoin formation (then thought to be a polymer) [21]. Later it was shown that these drugs inhibit β-hematin formation brought about under abiotic conditions and that inhibition thus occurs by direct interaction between the drugs and Fe(III)PPIX [22,23]. Later Mungthin et al. demonstrated that these drugs depend on the release of heme from hemoglobin for their activity, since inhibitors of hemoglobin digesting proteases antagonize their activity [24]. Sullivan et al. also provided direct evidence of interaction of chloroquine with hemozoin in the food vacuole of *P. falciparum* by electron micrographic autoradiography [25]. The interaction of antimalarials with Fe(III)PPIX has been reviewed previously [26] and several reviews are available on the role of hemozoin inhibition in the action of antimalarials, so this will not be discussed further here [27–31]. However, it is worth noting that recent studies have also indicated that chloroquine inhibits hemozoin formation in *S. mansoni*, reducing parasite burden as well as egg deposition in mice [32], suggesting that hemozoin inhibition may also be a viable strategy for treatment of schistosomiasis. Recently hemozoin has also been garnering interest because of its possible role in the immunological