Biochimica et Biophysica Acta 1840 (2014) 2032–2041

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Malarial hemozoin: From target to tool

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ARTICLE INFO

Article history:
Received 27 August 2013
Received in revised form 26 January 2014
Accepted 10 February 2014
Available online 17 February 2014

Keywords:
Hemozoin synthesis
Hemoglobin degradation
Hemozoin structure
Hemozoin biophysical properties
Plasmodium falciparum

ABSTRACT

Background: Malaria is an extremely devastating disease that continues to affect millions of people each year. A distinctive attribute of malaria infected red blood cells is the presence of malarial pigment or the so-called hemozoin. Hemozoin is a biocrystal synthesized by *Plasmodium* and other blood-feeding parasites to avoid the toxicity of free heme derived from the digestion of hemoglobin during invasion of the erythrocytes.
Scope of review: Hemozoin is involved in several aspects of the pathology of the disease as well as in important processes such as the immunogenicity elicited. It is known that the once best antimalarial drug, chloroquine, exerted its effect through interference with the process of hemozoin formation. In the present review we explore what is known about hemozoin, from hemoglobin digestion, to its final structural analysis, to its physicochemical properties, its role in the disease and notions of the possible mechanisms that could kill the parasite by disrupting the synthesis or integrity of this remarkable crystal.
Major conclusions: The importance and peculiarities of this biocrystal have given researchers a cause to consider it as a target for new antimalarials and to use it through unconventional approaches for diagnostics and therapeutics against the disease.
General significance: Hemozoin plays an essential role in the biology of malarial disease. Innovative ideas could use all the existing data on the unique chemical and biophysical properties of this macromolecule to come up with new ways of combating malaria.

1. Introduction

Malaria is a disease caused by *Plasmodium* parasites, resulting in approximately one million deaths every year around the world. Regions and countries affected by poverty are most at risk of infection. One of the greatest obstacles in the control of malaria has been the spread of drug resistance almost worldwide. Currently, the standard treatment of uncomplicated malaria in these regions consists of artemisin-based combination therapies (ACTs), while chloroquine combined with primaquine is the treatment of choice for chloroquine-sensitive infections. For the treatment of severe malaria there are two classes of drugs available: the cinchona alkaloids (quinine and quinidine) and artemisin derivatives (artesunate and artemether) [1]. Resistance to chloroquine and sulfadoxine–pyrimethamine fueled the ongoing scourge of *Plasmodium falciparum*, the principal cause of malaria morbidity and mortality in the world. In response to the increased number of infections, the World Health Organization (WHO) has recommended the use of combinatorial therapies that include artemisinin derivatives as first-line therapy. Nevertheless, even combinations with new drugs have the potential to create resistance [2–4]. Research efforts are needed to find alternative treatments for malaria that avoid the problem of drug resistance altogether.

One approach for the development of new treatments against malaria is to study the *Plasmodium* distinctive molecule hemozoin and to try to target this vital pathway of the parasite [5]. Hemozoin (HZ) is a metabolically crystallized byproduct of the digestion of hemoglobin by the parasite during infection of the red blood cells (RBCs). The formation of hemozoin from heme residues is common among diverse hematophagous organisms without phylogenetic relation to *Plasmodium spp*, such as *Schistosoma mansoni* and *Rhodnius prolixus* [6]. Since sequestration of heme into hemozoin is an essential process for the survival of the malaria and other apicomplexan parasites, this molecule has become an attractive target for new drugs that could interfere with the biocrystallization of hemozoin and would help fight diseases caused by these pathogens, especially malaria [7–10].

Abbreviations: HZ, hemozoin; RBC, red blood cell; FV, food vacuole; HDP, Heme Detoxification Protein; NMR, nuclear magnetic resonance; PPIX, protoporphyrin IX; CQ, chloroquine

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http://dx.doi.org/10.1016/j.bbagen.2014.02.009
0304-4165/

2. Hemozoin synthesis

2.1. Hemoglobin degradation

Hemoglobin is the principal component of red blood cells, composing approximately 95% of the proteins of the cytosol, reaching a concentration of about 5 mM inside the cytoplasm [11].

During the intra-erythrocytic stages of malaria infection, up to 80% of the cytoplasm of the host is consumed. Although during the ring stage some hemoglobin degradation is detectable, the major part of this process takes place during the trophozoite and schizont stages, which perform most of the metabolic activity of the parasite [12].

The hemoglobin degradation process occurs mostly inside the digestive vacuole of the parasite (Fig. 1). These organelles in *P. falciparum* are acidic structures with an estimated pH of 5.0 to 5.4 [13,14]. The acidic pH in these vacuoles is maintained by a proton gradient activated by an ATPase pump. It is thought that the digestive vacuole is a vesicle dedicated almost exclusively to hemoglobin degradation because of the lack of the characteristic lysosomal phosphatase and glycosidases present in other organisms. Hemoglobin degradation was believed to play a vital role as an amino acid source for malaria parasites because it has been observed that they have a limited capacity to synthesize their own. Some have alleged that the parasites do not need to degrade other macromolecules to sustain growth and development because they obtain most of the nutrients directly from the digestion of hemoglobin [15]. But, given that hemoglobin is a poor source of methionine, cysteine, glutamine and glutamate, and completely lacks isoleucine, parasites must be degrading hemoglobin for purposes other than just nutrition or detoxification. Such an idea was tested in experiments where parasites were placed in a culture medium that provided them with the 20 essential amino acids; it was shown that the parasites were still degrading hemoglobin [16]. Several studies demonstrated that some protease inhibitors block the proteolysis of hemoglobin, and the result was that the parasite development was interrupted, even with all nutritional resources available [17–20]. Therefore the idea of hemoglobin being digested mainly for nutritional purposes was not completely accurate. An equilibrium system exists inside the parasite between host hemoglobin degradation, efflux of the amino acids produced by hemoglobin degradation, and influx of extracellular amino acids and subsequent incorporation into parasite proteins. While the parasite grows and develops, the amount of hemoglobin degradation increases but not the amount of parasite protein: even though malaria parasites digest more than 65% of the host cell hemoglobin, they only use approximately 16% of this digestion to synthesize proteins for their own needs [21]. The membrane of the infected erythrocyte becomes more permeable to amino acids as it develops, allowing for large amounts of them to exit from the cell [21]. Later findings evidenced that the parasite digests the erythrocyte hemoglobin in order to prevent it from early lysis, which could take place if the parasite did not offset the increase in cell volume [22]. This colloid osmotic hypothesis was corroborated with mathematical models, which accurately fit the experimental data [23].

Most of the enzymes implicated in the hemoglobin degradation pathway have been elucidated. Two aspartic proteases (plasmepsins I and II) and a cysteine (falcipain) protease have been isolated and purified from the digestive vacuole of *P. falciparum*. All of these proteases function optimally at a pH of around 5, coinciding with the digestive vacuole acidity [24,25]. Aspartic proteases account for 60–80%, and cysteine protease for 20–40% of the globin-degrading activity of purified digestive vacuoles [11].

Plasmepsin I makes the initial attack between the α33Phe and 34Leu residues of hemoglobin and then the subsequent cleavages take place elsewhere. The 33–34 bond is located in a specific region that plays an essential conformational role in the hemoglobin molecule, acting like a hinge in the tetramer. Apparently, cutting the molecule in this specific site allows for the uncoiling of hemoglobin, exposing other sites for later proteolytic attacks by cysteine and aspartic proteases of the degradation pathway [25].

For the hemoglobin degradation process, the parasite needs both plasmepsins I and II. The reasons for the parasite needing two different enzymes for a single function are still unknown. In any case, different enzymatic expression patterns of these molecules have been detected along the intraerythrocytic stage of the parasite [26]. During the ring stage only plasmepsin I is expressed, and its expression continues during the following stages of the parasite. In contrast, plasmepsin II is mainly expressed during the trophozoite stage, which is the most metabolically active. Not surprisingly, almost all of the host cytoplasm is consumed during this stage. In fact, both enzymes are active during the trophozoite stage and are necessary to fulfill the massive catabolic processes taking place in this period of growth of the parasite [11].

In studies performed by Mueller and colleagues, exploiting the difference in sensitivity of both plasmepsin enzymes to the plasmepsin inhibitor SC-50083, they determined their role in the survival of the parasites, and the team concluded that blocking the activity of plasmepsin I leads to the death of parasites. Surprisingly, although the amount of plasmepsin II

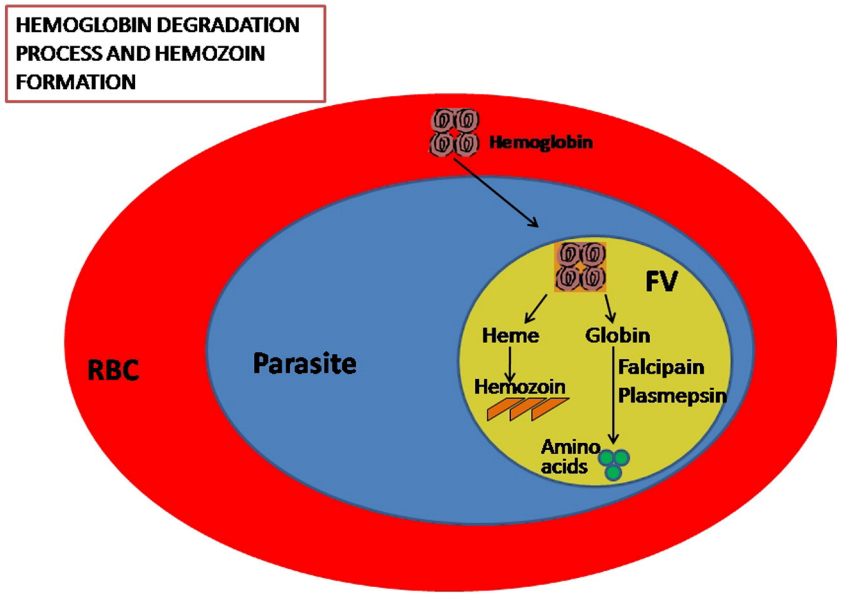

Fig. 1. Diagram of hemoglobin degradation and formation of hemozoin inside *P. falciparum* infected erythrocytes. Hemoglobin is degraded to obtain amino acids and to regulate osmotic pressure, and the parasite converts the toxic heme part into harmless hemozoin inside the parasitophorous food vacuole. RBC: red blood cell; FV: food vacuole.