The Digestive Vacuole of the Malaria Parasite

Subjects: Parasitology

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The malaria parasite resides within erythrocytes during one stage of its life cycle. During this intraerythrocytic period, the parasite ingests the erythrocyte cytoplasm and digests approximately two-thirds of the host cell hemoglobin. This digestion occurs within a lysosome-like organelle called the digestive vacuole. Several proteases are localized to the digestive vacuole and these proteases sequentially breakdown hemoglobin into small peptides, dipeptides, and amino acids. The peptides are exported into the host cytoplasm via the chloroquine-resistance transporter and an amino acid transporter that has also been identified on the digestive vacuole membrane. The environment of the digestive vacuole also provides appropriate conditions for the biocrystallization of toxic heme into non-toxic hemozoin by a poorly understood process. Hemozoin formation is an attribute of *Plasmodium* and *Haemoproteus* and is not exhibited by other intraerythrocytic protozoan parasites. The efficient degradation of hemoglobin and detoxification of heme likely plays a major role in the high level of replication exhibited by malaria parasites within erythrocytes. Unique features of the digestive vacuole and the critical importance of nutrient acquisition provide therapeutic targets for the treatment of malaria.

Plasmodium malaria digestive vacuole food vacuole endocytosis lysosome endosome hemozoin hematozoans anti-malarial

1. Introduction

Malaria is a common human disease in the tropics that exhibits substantial morbidity and mortality. The causative agent of malaria is a protozoan pathogen in the genus *Plasmodium*. *Plasmodium* and related Haemosporida (Apicomplexa) are dixenic parasites that infect vertebrate hosts, which include reptiles, birds, and mammals, and are transmitted by dipteran vectors [1]. A major characteristic of haemosporidians is an intraerythrocytic stage during the infection of the vertebrate host which is characterized by substantial proliferation. Residence within erythrocytes has certain advantages such as immune system avoidance and facilitation of vector transmission via blood-feeding arthropods. However, the erythrocyte may not be very accommodating from a nutritional perspective due to its rather simple composition and relatively low metabolism. The erythrocyte is approximately 95% hemoglobin and does not express the full gambit of metabolic pathways found in most cells. This raises questions about how intraerythrocytic parasites can exploit their host cell to obtain sufficient metabolites for survival and reproduction. And in some cases, this reproduction is quite notable with 30–40 progeny being produced in the order of days.

2. Life Cycle

The physiology of intraerythrocytic parasitism has been best studied in mammalian malaria parasites, and especially in *P. falciparum* due to its importance as a human pathogen and the ability to culture this parasite in vitro. The malaria parasite exhibits a complex life cycle involving mosquito transmission and a transient infection of the liver before infecting erythrocytes [2]. Infection of erythrocytes is initiated by merozoites which are initially released from infected liver cells. After invading erythrocytes, parasites undergo a trophic period characterized by parasite growth. The early trophozoite stage is often called the ring stage which lasts approximately half of the erythrocytic stage replicative cycle. The late trophozoites continue to increase in size and subsequently develop into stages called schizonts. The beginning of schizogony is marked by nuclear replication without cytoplasmic division resulting in the formation of multinucleated schizonts. Schizonts divide by a segmentation process to produce numerous merozoites that are released by rupture of the infected erythrocyte. These newly released merozoites reinitiate the intraerythrocytic replicative cycle after invading new erythrocytes to produce a chronic infection that often lasts for months. The repeated rounds of blood-stage schizogony are responsible for the clinical manifestations and pathology of the disease. Some of the merozoites, instead of undergoing asexual replication, develop into sexual forms called gametocytes. Mature gametocytes are uninucleated parasites that are infective to mosquitoes and play a key role in transmission.

During the intraerythrocytic stage, the parasite ingests the host cell cytoplasm and essentially converts the mass of the erythrocyte into its own mass. Thus, mature schizonts and mature gametocytes nearly fill the entire erythrocyte cytoplasm. During these maturation processes, approximately 70% of the soluble content of the infected host erythrocyte is ingested by the parasite [3]. Ingestion of the erythrocyte cytoplasm, which is primarily hemoglobin, is mediated by endocytosis and the endocytosed material forms the digestive vacuole. Within the digestive vacuole, also called the food vacuole, hemoglobin is broken down into amino acids which can then be utilized by the parasite [4][5]. The parasite also induces nutrient channels on the infected erythrocyte for the acquisition of metabolites [6][7].

3. Endocytosis and the Digestive Vacuole

Endocytosis is a general term referring to the uptake of substances that involves surrounding the material to be taken up with the plasma membrane and the enclosure of that material into membrane-bound vesicles. The engulfment of large particulate matter is called phagocytosis, and pinocytosis has historically been used to describe the engulfment of fluid. There are distinct types of fluid-phase endocytosis based on the size and volume of material being taken up and the mechanisms involved in the formation of endocytic vesicles [8][9]. For example, a major form of endocytosis is clathrin-mediated endocytosis which has historically been called receptor-mediated endocytosis [10]. Clathrin-independent endocytosis is poorly defined in regards to molecular components and their nomenclature is imprecise with various names [11]. Endocytic vesicles can fuse to form the endosome, or the endocytic vesicles can fuse with a pre-existing endosome [12][13]. Endosomes direct their content to other subcellular compartments, such as lysosomes. Alternatively, lysosomes can fuse with endosomes to form the lysosomal compartment.

The endocytic uptake of host erythrocyte cytoplasm was described in the early days of electron microscopy of the malaria parasite, and it was correctly surmised that this endocytosis was related to the digestion of hemoglobin [14]. Endocytosis producing small vesicles commences during the ring stage shortly after the parasite invades the host erythrocyte [15]. The intraerythrocytic parasite is surrounded by a membrane partially of host origin [16][17], called the parasitophorous vacuolar membrane (PVM). Therefore, endocytosis results in double-membrane vesicles with the inner membrane originating from the PVM [18]. The inner membrane rapidly disappears and initially the vesicles function as individual digestive vacuoles [19]. As the parasite grows and develops, the small independent digestive vacuoles coalesce into larger digestive vacuoles (**Figure 1**). The timing of this coalescence varies according to species with some species forming a large digestive vacuole during the early trophozoite stage, whereas in other species the large digestive vacuole appears in the late trophozoite stage [4]. In addition, it has long been recognized that the small digestive vacuoles do not coalesce into larger digestive vacuoles in the gametocytes, but remain dispersed as small vesicles [20].

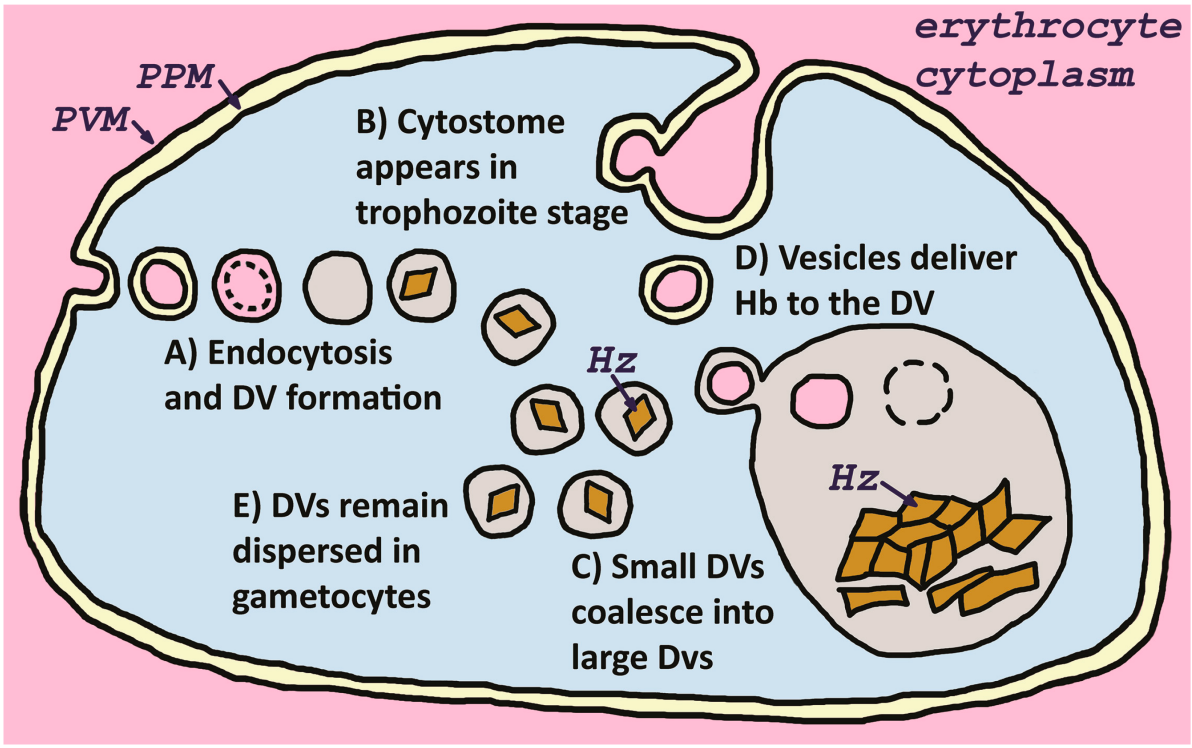

Figure 1. Endocytosis and digestive vacuole formation. Host cell cytoplasm is taken up by fluid-phase endocytosis involving the parasite plasma membrane (PPM) and the parasitophorous vacuolar membrane (PVM) leading to the formation of double membrane vesicles (A). The inner membrane, corresponding to the PVM, is rapidly degraded and the vesicles form small digestive vacuoles (DV) in which hemoglobin is degraded and hemozoin (Hz) forms. As the parasite matures, cytostomes appear and these serve as a major site for endocytosis (B). Also, as the parasite matures the small dispersed digestive vacuoles coalesce into large digestive vacuoles (C). Thereafter, hemoglobin (Hb)-containing vesicles deliver their content to the large digestive vacuoles (D). In gametocyte stages the digestive vacuoles do not coalesce and remain dispersed in the parasite cytoplasm (E).

Coincident with the maturation of trophozoites is the formation of cytostomes [21]. Cytostomes are tube-like openings on the parasite surface that serve as the focal points of endocytosis. On average, two and a half cytostomes are found per parasite [22]. Double-membrane vesicles are pinched from the base of the cytostome and these vesicles fuse with the digestive vacuole releasing the inner vesicle formed from the PVM. This inner membrane is rapidly degraded to release the hemoglobin. Other membrane invaginations have been described on the parasite surface and there may be multiple mechanisms of host cytosol uptake [23].

4. Hemoglobin Catabolism

The *Plasmodium* digestive vacuole contains numerous endo- and exo-proteases, and hemoglobin is broken down by the sequential action of these proteases in an ordered process [24]. In addition, gene knockout studies have demonstrated that no single food vacuole protease is essential, indicating functional redundancy [25]. The various endo-proteases participate in an ordered digestion of hemoglobin into large peptides, medium peptides, and small peptides. The initial cleavage likely occurs between residues 33 (phenylalanine) and 34 (leucine) of the α-subunit by a plasmepsin [26]. This proteolytic site is called the hinge region and is a highly conserved domain responsible for holding the hemoglobin tetramer together. Cleavage at this site likely results in an unraveling of the hemoglobin molecule and the exposure of additional proteolytic sites which leads to further degradation to medium-sized peptides by the plasmepsins and falcipains. Degradation of medium-sized polypeptides is mediated by falcilysins, and small polypeptides are degraded into dipeptides and amino acids by exopeptidases in digestive vacuole. The final products of these various proteases and peptidases are small peptides consisting of 5–10 amino acids, dipeptides, and amino acids that are transported to the cytoplasm of the parasite. Neutral aminopeptidases in parasite cytoplasm convert the peptides and dipeptides into amino acids [27].

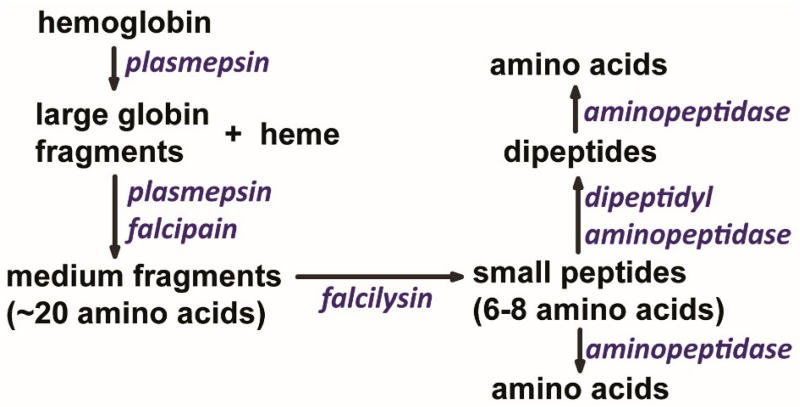

Figure 2. Ordered degradation of hemoglobin within the digestive vacuole. Initially, the combined actions of plasmepsins and falcipains break globin into medium-sized fragments that are subsequently broken down into small fragments by falcilysin. Following degradation by these endopeptidases, some of the small peptides are converted to dipeptides and amino acids by exopeptidases. Thus, the end-products of hemoglobin digestion are small peptides, dipeptides, and amino acids which are translocated to the parasite cytoplasm.

It is generally presumed that hemoglobin is digested to supply amino acids for the synthesis of parasite proteins. However, less than one-fifth of the amino acids obtained from the digestion of hemoglobin are incorporated into parasite proteins [28], and large amounts of amino acids are effluxed into the host erythrocyte [29]. Possible explanations for this discrepancy between the amount of hemoglobin ingested and amino acid utilization might be explained as a means to meet the space requirements of the growing parasite [30]. In addition, digestion of hemoglobin may help balance the intracellular osmotic pressure and thereby prevent premature lysis of the host erythrocyte [31]. Presumably, amino acids could also serve as an energy source through glucogenesis.

5. Anti-Malarials and the Digestive Vacuole

Many highly efficacious anti-malarial drugs target the digestive vacuole [5][32], and especially notable are 4-aminoquinolines, such as chloroquine, and artemisinin derivatives. Chloroquine has long been known to accumulate to high levels in the digestive vacuole. This accumulation may be due in part to the weak base properties of chloroquine and its subsequent protonation in the acidic digestive vacuole. The accumulation of chloroquine in the digestive vacuole may also be due to its binding to hemozoin [33][34]. Regardless of the mechanism(s), chloroquine is concentrated to millimolar levels within the digestive vacuole [35] and interferes with hemozoin formation [36][37]. Specifically, chloroquine may bind to the hemozoin crystal and thereby prevent the further addition of β-hematin dimers to the growing crystal [38]. This drug-mediated failure to detoxify heme increases oxidative stress, lyses membranes, and leads to parasite death.

Artemisinins are potent prodrugs that require activation [39]. The first step in this activation is the rapid and non-specific conversion of artemisinin derivatives to dihydroartemisinin. The second step is parasite specific and requires hemoglobin uptake and digestion [40]. Free heme in the digestive vacuole interacts with artemisinin and this interaction generates free radicals [41]. The activated artemisinin alkylates heme and prevents it from forming hemozoin crystals [42]. As with the 4-aminoquinolines, artemisinin derivatives also block heme detoxification and increase oxidative stress in the parasite resulting in parasite death. Heme-mediated activation of artemisinin also results in widespread parasite protein alkylation [43]. This would contribute to parasite death by inhibiting key proteins in essential parasite pathways.

6. Other Intraerythrocytic Protozoan Parasites

Intraerythrocytic protozoa are only found in the Apicomplexa [44]. The four groups of apicomplexans exhibiting intraerythrocytic stages are haemosporidians, piroplasmids, haemogregarines, and haemococcidians. Haemosporidians and piroplasmids form a group called hematozoa and haemogregarines and haemococcidia are within the coccidian clade—a sister group of the hematozoans. By far, most of the knowledge on feeding mechanisms within the infected erythrocyte is from mammalian malaria parasites which are members of the haemosporidian clade. Several haemosporidians produce hemozoin and the presence of hemozoin is used as a taxonomic tool within this group [45]. The ability to form hemozoin emerged only once and is found in the *Plasmodium* and *Haemoproteus* genera (**Figure 3**). This emergence of hemozoin formation may have occurred

between 57 and 69 million years ago [46]. There are no reported losses of hemozoin production within this clade indicating it is a robust biological advantage. Hemozoin formation is not found in the piroplasmids, haemogregarines, or haemococcidians, suggesting that these species do not extensively digest hemoglobin or have other mechanisms to detoxify heme. This gain of hemozoin formation in just one clade of erythrocyte-infecting protozoa strongly suggests a genetic basis for hemozoin formation. Identification of the gene(s) involved in hemozoin formation may provide additional therapeutic targets for the treatment of malaria.

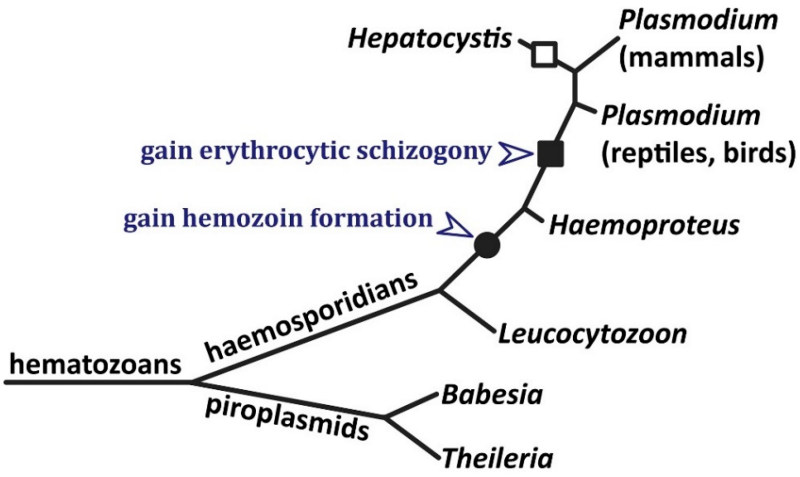

Figure 3. Phylogenetic relationships among the hematozoans and the gain of hemozoin formation. Hematozoans are characterized by one stage of the life cycle involving the infection of erythrocytes. The phylogenetic tree shows probable branching order of major genera [45][47]. Branch lengths do not depict evolutionary distances and branches do not depict complexity of the genera. *Hepatocystis* may branch within the mammalian *Plasmodium* clade [1]. The filled circle shows the gain of hemozoin formation, and the filled square shows the gain of schizogony during the erythrocytic stage. Erythrocytic schizogony is lost in *Hepatocystis* (open square).

Although probably not related to hemozoin formation, schizogony during the erythrocytic stage emerged after hemozoin production and is used to distinguish *Plasmodium* from *Haemoproteus* [45]. Erythrocytic stage schizogony has been lost in *Hepatocystis*.

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