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HAEMOGLOBIN DIGESTION: SOURCE OF AMINO ACID, IRON (Fe) AND A GOOD SURVIVAL STRATEGY FOR *Plasmodium falciparum*

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Abstract

Plasmodium falciparum like any other living organisms requires nutrients and other vital minerals especially Iron (Fe) and Amino acid for it growth and development. Therefore, the aim of this paper was to review the state of knowledge on the haemoglobin digestion as a cheap source of amino acid and iron and its contribution to the success of *Plasmodium falciparum's* survival. Haemoglobin comprises 95% of the cytosolic protein of the red blood cell, therefore Hemoglobin digestion is the major catabolic process through which *Plasmodium falciparum* produces the much needed nutrient .The haemoglobin digestion is carried out in Digestive Vacoule under the influence of: aspartic, cysteine and metallo proteinases. The process begins with the ingestion of the host cell haemoglobin into the digestive vacuole through cytosome. It begins with an initial proteolytic cleavage which occurs at the hinge region of the alpha globin chain and is mediated by the aspartic proteases: plasmepsins I, II, and IV. Plasmepsins and falcipain-2 and 3, carry out further degradation of the denatured globin. The resulting small globin peptides serve as the substrates for falcilysin. In addition, a Dipeptidyl Aminopeptidase (DPAP) removes dipeptide from the Ntermini of the peptide generated. The amino peptidases then

KEYWORDS:
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convert these to amino acid. Heme is also Produced but it is detoxified and expel ,while the excess heme is decomposed to release free Iron. Apart from amino acid generation, haemoglobin degradation provided other means of survival strategies for parasite. This critical step should be targeted toward blocking the spread of *Plasmodium falciparum.*

INTRODUCTION

Amino acid and Iron (Fe) are essential nutrient and element respectively for virtually all forms of life. This metallic compound is needed for the catalysis of DNA synthesis and for a variety of enzymes concerned in electron transport and energy metabolism (Bruno et.al.,2001). Hemoglobin, being by far the most abundant reservoir of iron within humans, is thus an attractive nutrient iron source for invading pathogens like *Plasmodium falciparum* (Gleb and Eric, 2012). For that, malaria parasite spends most part of its life cycle inside the red blood cells of its human host. These terminally differentiated cells provide protection from the host's immune system, but pose logistical difficulties with respect to obtaining nutrients needed for growth and for the disposal of waste products (Nectarios et.al.,2007).Therefore, digestion of Hemoglobin is a major catabolic process of *Plasmodium falciparum* in merozoitic stage (Kondapalli, et.al.,2012) which produces the much needed nutrient required for the survival of the parasite. Malaria parasite also requires amino acids for the synthesis of its proteins, these molecules and energy are used to maintain the homeostasis, growth and reproduction of the parasite. The three sources of amino acids are: de novo synthesis, import from host plasma, and digestion of host hemoglobin. Hemoglobin comprises 95% of the cytosolic protein of the red blood cell, where it is present at a concentration of 5mM. During the intraerythrocytic cycle, the host cell cytoplasm is consumed and an estimated 60–80% of the hemoglobin is degraded to support parasite growth and asexual replication during this stage. The bulk of hemoglobin degradation occurs via a semi ordered process by proteases contained within the lysosome-like organelle of the parasite, termed the food vacuole (Michelle, et.al.,2008), which is internally

acidic (Juan and Neira,2012), and it is the site where the actual haemoglobin digestion and heme detoxification are carried out (Nectarios et.al., 2007). In *P. falciparum*, some hemoglobin degradation is seen during the ring and early schizont stages of development, but the vast majority of this digestion is occurring during the trophozoite stage, that is 18 to 32hours after invasion (Milani et.al.,2015).

MATERIALS AND METHODS

Literature Search

The following data bases were visited; PubMed,Web of Knowledge, EMBASE, Web of Science,
Scopus, Google scholar, World Health Organization's WHOLIS and Medline in order to obtain reliable and authentic research and published articles on the topic.

Search Terms Used

Search terms that were directly or indirectly linked to Haemoglobin digestion by *Plasmodium falciparum* were used in order to generate relevant and authentic research papers. The following search terms were specicically used; Haemoglobin, Haemoglobin digestion by *Plasmodium falciparum*, source of iron, haemoglobin ingestion, survival strategies and successes, Proteinases, aspartic, cysteine and metallo proteinases, Infected Red blood cell, Mechanism of haemoglobin digestion, Digestive vacuole and it role in haemoglobin digestion, Cytosomes, heme detoxification, internalization of hemoglobin, invagination of the parasitophorous vacuolar membrane, catabolism of haemoglobin, de novo synthesis, import from host plasma and concentration of the haemoglobin. All the search terms used were directly associated with *Plasmodium falciparum* in order to generate relevant research papers and articles. All research papers generated were carefully and critically analyzed and scrutinized, after which the papers were grouped and classified based on the various headings and Subheading of the title of the review topic.

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HEMOGLOBIN INGESTION

A prerequisite for complete hemoglobin digestion is the uptake and transport of host cell's hemoglobin to the Digestive Vacoule (DV) (Michelle, et.al.,2008), Therefore, during its 48-hour residence within an infected red blood cell, an asexual *Plasmodium* parasite imports or ingests up to 80% of the host hemoglobin into the acidic digestive vacuole (Paul and Daniel,2014). The transport of hemoglobin from the host erythrocyte cytosol to the parasite Digestive Vacoule is a long-known but poorly understood biological process. While in the trophozoites stage, the parasite avidly ingest and degrade host erythrocyte hemoglobin, the internalization of hemoglobin is thought to occur through an unusual and specialized structure, the cytostome (Daniel,1990). In this, small portions of cytoplasm are taken up by micropinocytosis, and as the parasite matures, a larger volume of hemoglobin is ingested by means of a cytostomal system that is formed by invagination of the parasitophorous vacuolar membrane and the parasite plasma membrane (Susan et.al., 1997).

A cytostome is defined as a localized invagination of the parasite's outer membranes (the parasitophorous vacuolar membrane) and the parasite plasma membrane (Milani et.al., 2015),in some cases several cytostomes can be formed. When the cytostomes ingest red cell cytoplasm, double membrane-delimited vesicles are formed by budding. The hemozoin-containing vesicles appear to fuse, forming one or two large, single membrane-enclosed digestive vacuoles that contain a cluster of hemozoin crystals. The number and the size of the hemozoin crystals in the erythrocyte depend on the stage of the parasite development, with the least amount of the hemozoin detected in the ring stage and the highest amount in the schizont stage (Lee et.al,2006).

Below(Figure 1) is the schematic diagram of haemoglobin ingestion by the *Plasmodium falcifarum.*

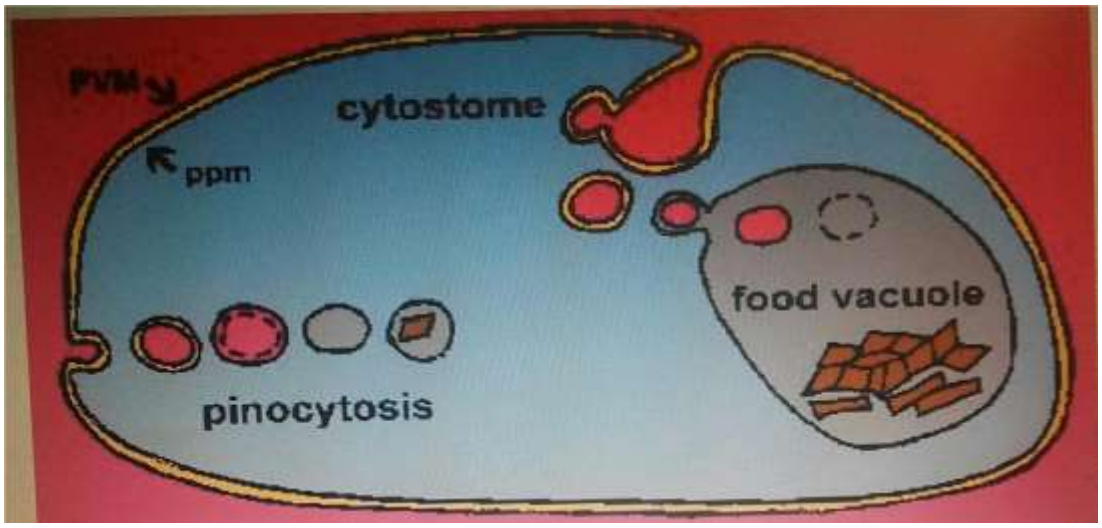

FIGURE 1: Ingestion of the host's cytoplasm by the parasite, Source; Hangjum et.al(2014)

MECHANISM OF HAEMOGLOBIN DIGESTION

In blood stages, malaria parasites consume most of the hemoglobin inside the infected erythrocytes (Hangjun et.al.,2014).Malaria parasites are evolutionarily equipped with intricate machineries to degrade host haemoglobin (Hb) during their intra-erythrocytic stages of development (Patrath et.al.,2016). In *P. falciparum*, hemoglobin is degraded in a single, acidic organelle known as the food vacuole or digestive vacuole. Haemoglobin molecules, taken up by endocytosis undergo hydrolysis in the parasite's digestive acidic vacuole under the influence of Cysteine , aspartic and metallo proteases (Paul, 2015), where the aspartic proteinases is responsible for initiating the haemoglobin digestion (David and Colin,2002).These proteolytic enzymes have pH optima ranging from 4.5-5.0, thus, confirming an acidic environment of the Digestive Vacuole (Ibrahim and Abubakar, 2019).

The initial stages of hemoglobin catabolism have been well characterized and involve a diverse set of endoproteinases. Three classes of proteases include Plasmepsin (Aspartic), Falcipains (Cysteine) and Falcilysin (Metallo), these participated actively in the degradation of hemoglobin. The initial proteolytic cleavage occurs at the hinge region of the alpha globin chain and is mediated by the aspartic proteases plasmepsins I, II, and IV (Michael

et.al.2004).Plasmepsins, and a family of cysteine proteases, falcipain-2 and 3, then carry out further degradation of the denatured globin.

The resulting small globin peptides serve as the substrates for falcilysin (Christiana and Daniel, 2003).

The proposed pathway of hemoglobin digestion involves an initial cleavage of the haemoglobin by plasmepsins-1 and possibly falcipain-2. The peptide fragments produced by these digestions are then digested in to smaller peptide by falcilysin. In addition , a dipeptidyl aminopeptidase (DPAP) activity has been identified within the food vacuole. It is postulated that the DPAP may remove dipeptide from the N-termini of the peptide generated through the action of various endopeptidases in the food vaoule and the amino peptidases can convert these amino to amino acid. Amino acids derived from hemoglobin catabolism are incorporated into plasmodial proteins and parasites can rely on hemoglobin catabolism to supply sufficient quantities of all amino acids except those five that are rare in or completly absent (Methionine,Cysteine, Glutamine, Glutamate and Isoleucine) from hemoglobin (Michael et.al.,2004). The schematic presentation of hemoglobin digestion is shown in the figure 2 below.

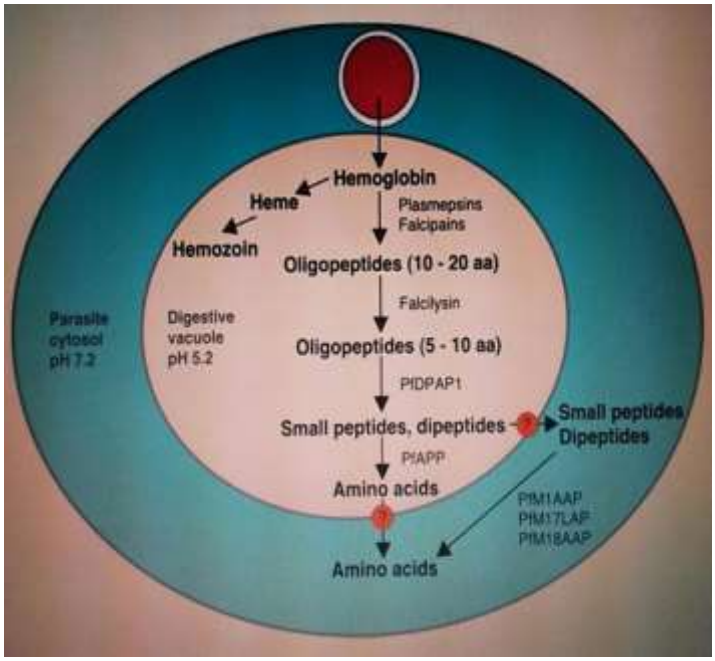

Figure 2:Schematic presentation of haemoglobin digestion, Source; Hangjum et.al(2014)

Of the total heamglobin ingested and degraded by the parasite, only 16% of the haemoglobin is use to release amino acids for protein biosynthesis. The excess is discharged out of the

infected red blood cells (IRBCs) to the surrounding plasma mainly through new permeation pathways (NPPs) of broad solute selectivity induced by the parasite in the host cell membrane (Virgilio et.al.,2003). This because, is to highly decrease the concentration of the haemoglobin as it causes fluid to move in by the process of osmosis, which may lead to the swelling, and subsequently bursting of the cell. For that the parasite digest large proportion of the haemoglobin than it requires for its own protein (Gunanidhi et.al.,2010).

FATE OF HEME GENERATED DURING HAEMOGLOBIN DIGESTION

Hemoglobin proteolysis releases soluble heme (Susan et.al., 1997; Thomas et.al.,2014) which accumulates in crystalline particles within the digestive vacuoles (Daniel et.al.,1990).The heme generated is highly toxic for the *Plasmodium* parasite, as such it is sequestered in the cell by conversion to an insoluble form known as hemozoin, or malarial pigment (Lee, et.al.,2006), the

Hemozoin (malaria pigment) is a crystalline dimer of β- hematin (ferriprotoporphyrin IX) (Ghazi,2017). The toxicity of the heme is due to its ability to bind and inhibit proper protein function (Paul and Daniel, 2014).To overcome the toxicity of free heme, malaria parasites crystallize heme by forming iron-carboxylate bonds between two heme molecules in a repeating arrangement, yielding inert hemozoin crystals. Hemozoin formation seems to be the dominant process used by malaria parasites to detoxify heme, as 95% of the heme released from Hb digestion is estimated to end up in hemozoin (Hangjun et.al.,2014). The remaining heme is decomposed by glutathione (GSH) in the cytosol, which produces free iron that can enter redox cycling and generate O^- in the parasite cytosol (Shin-ichiro, et.al.,2004).

CONCLUSION

Haemoglobin is cheap source of amino acid for the *Plasmodium falcifarum* and the amino acid is only generated in an ordered process under the influence of some proteinases. In addition, *Plasmodium falciparum* derived

other important benefit which include; protection against the host's immune system, it creates enough space in the cytoplasm and also decrease haemoglobin concentration, hence prevent inflow of fluid due to osmosis which may lead to the bursting of the entire infected red blood cells.

RECOMMENDATION

Haemoglobin digestion is very crucial to the survival of *Plasmodium falcifarum*. Therefore it is recommended that this step should be targeted in blocking the spread of the parasite through provision of antigens that will illicit good response by the production of large amount antibodies to attack the parasite at this crucial step.

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