The Malaria Pigment Haemozoin—A Focal Point of Action for Antimalarial Drugs

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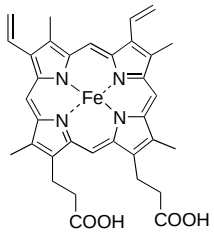
Although mankind has been aware of the origin and transmission of malaria for several centuries,[1–3] still an estimated 300 – 500 million people are affected globally each year with an annual death toll of at least 1.1 – 2.7 million mainly involving children and pregnant women. According to data from the World Health Organization (WHO), each day up to 3000 children under 5 years die of malaria with 90% of these cases occurring in sub-Saharan Africa. It is estimated that about one third of all hospital admissions and 25% of all deaths of children in this region are related to malaria. Nevertheless malaria is endemic in about 100 countries and is a significant problem in Brazil, Afghanistan, Sri Lanka, Thailand, Indonesia, China, Vietnam, and Cambodia, too.[4] The infection is caused by the bite of the female *Anopheles* mosquito[2] that leads to the transmission of a protozoan parasite of the genus *Plasmodium*.[3] Four species of *Plasmodium* can produce the disease in various forms, the most dangerous species being *P. falciparum*.

The infective cycle starts by infection with sporozoites that invade hepatocytes. After several days the progeny merozoites enter the blood stream and infect erythrocytes. Here several stages of asexual reproduction occur, resulting in the rupture of the erythrocyte and ultimately the formation of gametocytes. These can be ingested by mosquitoes and form a zygote initiating the sexual reproductive cycle, again producing sporozoites. If large numbers of parasites are present, the cell division and red blood cell destruction causes the well known symptoms of fever, shivering fit, and anemia.[5]

While statistics at a first glance might indicate that the global malaria situation has stabilized, an alarming increase in drug resistance and decrease in therapeutic efficacy of available drugs has been observed.[4] A drug-based therapy should lead to the elimination of the parasite in the different stages of its life cycle and to termination of erythrocyte destruction. The therapy depends on parasite sensitivity and drug availability. Common antimalarial agents that affect parasite metabolism are primaquine, several antibiotics, artemisinins, sulfonamides, antifols and chloroquine, and related compounds. The absence of adequate health services in endemic zones frequently results in a recourse to self-administration of drugs with incomplete treatment. The resulting drug pressure selectivity (and increasing vector efficiency) is a major factor in the increase in resistance of the parasite to effective drugs.[4]

A crucial step for possible drug interactions and medical problems involves parasite maturation in the red blood cells. The parasite proteolyzes ingested erythrocyte haemoglobin as its major nutrient source. The parasites digest up to 75% of host cell haemoglobin within a lysosomal organelle, the digestive vacuole. While this process delivers essential amino acids, it also liberates cytotoxic haem [ferriprotoporphyrin (**1**)]. As *Plasmodium* lacks haem oxygenase and cannot degrade haem by macrocycle cleavage, it uses a rather unique way for haem detoxification. For the haem to be sequestered, it is transformed by a parasite-specific aggregation process resulting in an inert form, called haemozoin or malaria pigment.[3, 6] Haemozoin forms large, insoluble crystals that after destruction of the red blood cells are deposited in lymphoid tissue, spleen, liver, bone marrow, and brain, resulting in organ pigmentation of the affected patients.[3, 7]



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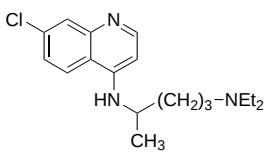

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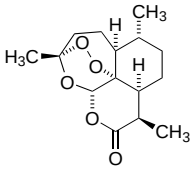

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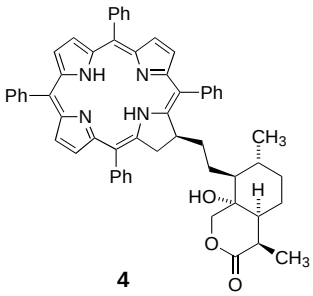

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A first step towards an understanding of the molecular structure of haemozoin was made by the observation that its chemical (elemental composition and solubility) and spectroscopic properties (IR, ESR, X-ray absorption, and Mössbauer spectra) are identical to so-called β-haematin.[8] β-Haematin is formed as an insoluble phase upon prolonged heating of acidic solutions of haematin or more conveniently by abstraction of HCl from haemin with non-coordinating bases under anhydrous conditions.[7, 9a] As final proof of the identity of haemozoin and β-haematin it was shown that the crys-

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tallographic unit cells of both materials were the same.[9b]

For some time it was assumed that this insoluble form was a polymer of Fe^{III}-protoporphyrin units. A carboxylate group oxygen atom of one propionate side chain was believed to coordinate to the iron center leading to a polymer with the general structure -(porphyrin)Fe^{III}-[OOC-CH_2-CH_2-(porphyrin)Fe^{III}]$_n$-OOC-. The other propionic acid group was thought to form hydrogen bonds with a different chain of propionate-linked haems.[8b, 9b]

However, the correct structure was recently revealed by Stephens, Bohle, and co-workers as an aggregate of hydrogen-bonded dimers.[10] For their study they used high-resolution X-ray powder diffraction data obtained with a microcrystalline powder of β-haematin. Rietveld refinement clearly showed the structure to consist of five-coordinated high-spin Fe^{III}-protoporphyrin dimers (Figure 1). Dimerization is achieved through η^1-coordination of a carboxylate group oxygen atom of a propionate side chain to the iron center of a porphyrin, which in turn uses a propionic acid chain for coordination. Thus, each dimer is held together by two Fe—O axial bonds. Crystallographically these dimers serve as the units for a hydrogen-bonded polymer. The propionic acid group of each porphyrin not involved in iron coordination forms a classic hydrogen-bonded diacid aggregate with another dimer, resulting in a hydrogen-bonded polymer with dimeric Fe^{III}-protoporphyrin subunits (Figure 1).

While numerous dimeric porphyrin structures are known that involve coordination of a peripheral substituent functionality with a central metal atom,[11a] haemozoin is the only crystalline protein-free porphyrin aggregate structure found in vivo.[11b] As haemozoin is the focal point of action for several antimalaria drugs, the detailed structure now available has important implications for finally understanding the antimalarial drug actions.

Malaria pigment is not formed spontaneously from haem or haemoglobin under physiological conditions and the (bio)chemistry of its formation remains a mystery. Several "active principles" have been associated with the acceleration of haemozoin formation in vivo. In vitro it can be achieved with parasite extracts[6a] or appropriate nucleation,[12a] whereas histidine-rich proteins or a putative haem polymerase enzyme[6a, 12a,b] and phospholipids[12c] have been implicated here as well.

Several antimalarial drugs act by interfering with haemozoin formation. For example, chloroquine (**2**) has been shown to bind to free ferriprotoporphyrin, and the complex thus formed then binds to the growing haemazoin aggregate, terminating extension and blocking further haem incorporation.[13] It appears that all antimalarial quinolines act in a similar fashion, requiring first formation of a haem – drug complex and then its binding to the haem aggregates, thereby blocking further haemozoin formation. Obviously, the formation of a highly ordered structure like that shown in Figure 1 is not possible when the growing face of the haemozoin crystallites is occupied by, for example, chloroquine – haem complexes.[14] As chloroquine – haem – haemozoin interaction involves purely chemical action, the known chloroquine resistance is the result of the drug not reaching the target site, that is, changes in the uptake process.[13]

As most older drugs have resistance problems, currently the most promising drug is artemisinin (Qinghaosu, **3**) an endoperoxide sesquiterpene lactone produced by an annual Chinese herb.[15] Artemisinin and its derivatives have the broadest stage specificity of all antimalarial drugs and causes structural changes in the erythrocyte stage of the parasite. Its peroxide function is vital for the drug action and its mode of action is believed to involve the formation of cytotoxic compounds such as free radicals and reactive aldehydes. It was generally believed that **3** reacts first with intraparasitic haem giving rise to oxyl radicals that rearrange to C-centered radicals[16] which are then able to alkylate proteins and DNA.[17] However, its exact mode of action is still unknown. Artemisinin is known to bind haem[18a] and has been shown to inhibit specific proteases.[18b] Recent chemical studies on artemisinin analogues also reinforce the hypothesis that the formation of tight iron protoporphyrin – artemisinin complexes, possibly through coordination of the peroxide group to the Fe^{II} center,[19] is involved in the activation of their antimalarial action.[20]

Recently, several new pieces of evidence have become available. Wu et al. showed that artemisinin can be activated by non-haem Fe^{II} and then react with the SH group of cystein, suggesting a possible mechanism for the observed alkylation of biomolecules.[21] Intriguingly, Meunier and co-workers showed that artemisinin is also capable of directly alkylating simple porphyrins such as (5,10,15,20-tetraphenylporphyrinato)manganese(II), giving rise to β-alkylated hydroporphyrins (e.g. **4**).[22, 20b] This reaction appears to be general for all antimalarial trioxanes. This is further evidence for the formation of C-centered radicals in the vicinity of haem which then alkylate and inactivate proteins involved in haemoglobin breakdown (haemoglobinases) and perhaps the histidine-rich protein,[12b] which may play a role in haemozoin formation. Further modes of action of **4** involve its ability to *meso*-hydroxylate haem and thus point to its potential involvement in haem degradation.[23] Pandey et al. showed that artemisinin not only binds haem and inhibits the haemozoin aggregation, they also observed a concentration-dependent artemisinin-induced breakdown of haemozoin in vitro.[18b] This combination of several actions aimed at proteins, haem, and haemozoin might be an explanation for the efficacy of this drug to counteract malaria. In any case, haemozoin has emerged as the focal point of action for several antimalarial

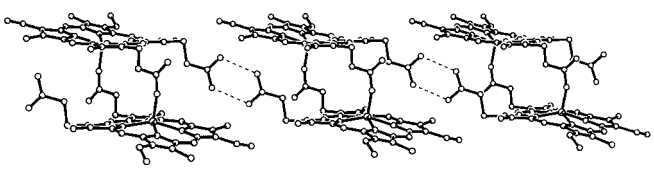

Figure 1. View of the aggregates formed by β-haematin in the crystal.[10]

agents with quite different chemical structures.

The observed increase of resistance against antimalarial drugs and ongoing problems in developing vaccines[24] or using bacteria as bio-weapons for mosquito vector control[4] mandates worldwide research efforts aimed at developing novel and drastically improved drugs. It can only be hoped that the breakthroughs made in recent years with the structural elucidation of haemozoin and the better understanding of the action of drugs such as artemisinin and chloroquine will speed up this process.

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[1] African fossils of the vector date back 30 million years, and reports on deadly fevers have been known since mankind began to document such cases. The oldest references are Chinese reports on malaria-like illnesses from 2700 B.C., the Vedic writings of 1600 B.C. in India, and reports by Hippocrates from Kos (4th century B.C.). Alexander the Great is believed to be an early prominent victim of malaria (323 B.C.). The first modern scientific studies were performed by Lancisi in Rome in 1717 who identified the connection between malaria ("bad air", a term coined in Italy in the 7th century) and mosquitoes.[2] The parasite of malaria was discovered by Laveran in 1880.[3]

[2] G. M. Lancisi, *De noxibus paludum effluvis, eorumque remedis*, Typis Jo. Mariae Salvioni, Romae, **1717**.

[3] C. L. A. Laveran, *Nature parasitaire des accidents de l'impaludisme: description d'un nouveau parasite trouvé dans le sang des malades atteints de fièvre palustre*, J.-B. Baillière, Paris, **1881**. For his discovery of the malaria parasite Laveran palustre, was awarded the Nobel Prize in physiology or medicine in 1907.

[4] "WHO Expert Committee on Malaria: 20th report", *W.H.O. Tech. Rep. Ser.* **2000**, *892*. For general information, see the World Wide Web pages of the WHO (Infectious Disease Section; www.who.int), the Multilateral Initiative on Malaria (www.mim.nih.gov), the Malaria Foundation International (www.malaria.org), and also www.malarianetwork.org and www.malariamedicines.org.

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