Unveiling interactions of the antimalarial drug chloroquine with haeme in aqueous solutions through spectroscopic and quantum mechanical methods

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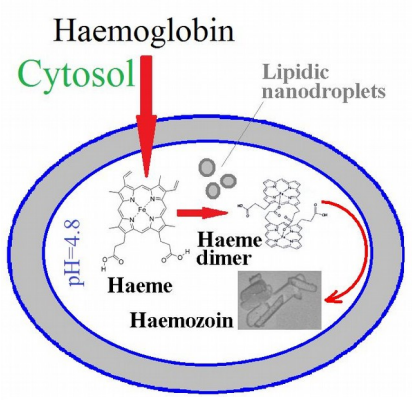

Figure 1. Probable detoxification pathway of free haeme in the *Plasmodium* food vacuole.

Malaria is one of the most worldwide spread parasitic disease. It is caused by *Plasmodium* protozoa, which eventually infect human erythrocytes and digest the host haemoglobin. This process releases free haeme (Fe-protoporphyrin-IX), which is toxic to the parasite as it produces reactive oxygen species (ROS), the cause of oxidative stress. The protozoon deactivates haeme by promoting its crystallization into solid pale-yellow hemozoin, that gives the characteristic skin color of malaria-infected people.

Aminoquinoline-type (AQ) drugs interfere with this detoxification process either by directly hampering haeme-haeme self-recognition in solution [i] or by preventing the growth of hemozoin crystals [ii] (Figure 1). The nature of the specific AQ compound-haeme scaffold interactions is not yet understood, even though it is a necessary requirement to explicate antiplasmodial activity.

We report here on an experimental and theoretical study of the AQ- type antimalarial chloroquine (CQ)-free haeme interactions in aqueous solutions. Extended X-ray Absorption Fine Structure (EXAFS) experiments at the Fe Kα absorption edge (7.1 keV) were performed at the BM26A station of the ESRF facility in Grenoble (FR) on various haeme-containing solutions, both in the presence and in the absence of CQ. The effect of pH was monitored through the addition of suitable buffers in the 4–7 range at variable pH interval. A tensioactive (sodium dodecyl sulfate) at its critical micellar concentration was also employed to model lipidic nanodroplets in the parasite food vacuole, as their presence was reported [iii] to favor hemozoin crystallization (Figure 1). EXAFS results were complemented by accurate UV absorption measurements of the same solutions and DFT B3LYP 6-311G(d,p) simulations of possible haeme:chloroquine adduct geometries.

We found evidence that, at least in the experimental conditions here employed, CQ does not set stacking π···π interactions with the protoporphyrin scaffold, even though this geometry was proposed as the most probable one through molecular mechanics simulations [iv,v] and previous EXAFS studies of mesohaematin anhydride in dimethylsulfoxyde [vi]. Rather, our DFT calculations point out that CQ and haeme seem to recognize each other through electrostatic interactions among lateral charged groups. If proven true, this would have obvious implications on the engineering of novel antimalarials able to thwart the parasite adaptability against classical AQ-based therapies.

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