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An atomic scale mechanism for the antimalarial action of chloroquine from density functional theory calculations

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Dedicated to the memory of Dr David R. M. Walton, Founding Editor, Transition Metal Chemistry

Abstract

The X-ray crystal structures of complexes between the antimalarial drugs quinine, quinidine and halofantrine and their biological target, iron(III) ferriprotoporphyrin IX (FePPIX), have been reported in the literature [de Villiers KA, Gildenhuys J, le Roex T (2012) ACS Chem Biol 7:666; de Villiers K, Marques HM, Egan TJ (2008) J Inorg Biochem 102:1660], and show that all three drugs utilize their zwitterionic alkoxide forms to coordinate to the iron atom via Fe-O bonds. In this work, density functional

theory calculations with implicit solvent corrections have been used to model the energetics of formation of these complexes. It is found that the cost of formation of the active zwitterionic form of each drug is more than offset by the energy of its binding to FePPIX, such that the overall energies for complexation of all three drugs with FePPIX are moderately favourable in water, and rather more favourable in *n*-octanol as solvent. The calculations have been extended to develop an analogous model for the complex between FePPIX and chloroquine, whose structure is not presently known from experiment.

Keywords

Chloroquine; Density Functional Theory; Ferriprotoporphyrin IX; Iron(III); Malaria

Introduction

Malaria persists as one of the most widespread infectious diseases, with an estimated 207 million cases and 627,000 deaths in 2012 [1]. Malaria prophylaxis and treatment utilize a range of drugs, many of which are increasingly limited in their efficacy by emerging resistance in one or more of the five species of *Plasmodium* parasites that are responsible for the disease. There is therefore an urgent need to develop new antimalarial drugs. Currently available drugs, including artemisinin (AM), quinine (QN), quinidine (QD), halofantrine (HN) and chloroquine (CQ), are all thought to act by disrupting the crystallization of iron(III) ferriprotoporphyrin IX ($FePPIX^{\dagger}$) to haemozoin within the parasite's digestive vacuole (Scheme 1) [2-7]. The parasite relies on this process to detoxify FePPIX, which it releases in large quantities as it digests the host's haemoglobin within the host's red blood cells. Failure to crystallize FePPIX as haemozoin results in an increase in the solution concentration of free FePPIX, which is fatal for the parasite. Crystallization of FePPIX is promoted in the digestive vacuole by a combination of low pH (*ca.* 4.5-4.9) and a lipid-rich environment [8-11]. All of the drugs listed above are able to prevent haemozoin crystal growth, although the exact mechanisms are evidently somewhat different for each class of drug. Thus, AM is known to alkylate the *meso* positions of FePPIX, preventing addition of the modified haem to the crystals [2]. In contrast, the X-ray crystal structures of adducts of QN, QD and HN with FePPIX show that all three drugs act as alkoxide ligands for the iron(III) centre [3,4]. Overall charge neutralization in each complex is achieved by the drug adopting a zwitterionic structure, in which a proton is transferred from the alcohol to the tertiary amine (Scheme 2, step **A**). The NH^{+} moiety then participates in hydrogen bonding to the anionic propionate group of the porphyrin (step **B**).

<Schemes 1 and 2 about here>

Chloroquine was first prepared and investigated as an antimalarial in 1934, and has saved countless lives since that time [12]. Although CQ also disrupts the growth of haemozoin crystals, the molecular basis of its action has remained obscure. Solution studies have revealed that CQ associates with FePPIX and its synthetic analogues *in vitro*, but only by inherently weak non-covalent interactions [13-15]. Egan and co-workers have carried out a number of recent studies, which show that whilst CQ can induce formation of the μ-oxo dimer of FePPIX in solution [16], the key effect of CQ is probably the inhibition of crystallization of FePPIX by adsorption of the drug onto the most rapidly growing crystal faces of haemozoin [7,17]. Nevertheless, no X-ray crystal structure of a CQ-FePPIX complex has been reported to date. Recently, we have calculated the binding energies of a range of ligands with FePPIX and its parent iron(III) porphine by density functional theory (DFT) quantum calculations with implicit solvent corrections for both water and *n*-octanol, the latter solvent being included in order to model the lipid-rich environment of the parasite's digestive vacuole [18]. The results of these calculations confirmed that none of the functional groups available to CQ can bind to the iron(III) centre with anything approaching the affinity of the alkoxide ligand present in the zwitterionic forms of QN, QD and HN. Therefore, the paradox of CQ action remains; somehow, it can effectively disrupt haemozoin crystallization, but it lacks any functional groups capable of strong direct interactions with FePPIX. In this communication, we propose a resolution of this conundrum, based on the results of comparative DFT calculations on both the known complexes of QN, QD and HN with FePPIX, and the unknown complex of CQ with FePPIX.

Computational methods

All DFT calculations were performed with Gaussian09 software [19]. Input geometries were taken from the X-ray crystal structures [3,4] or created using Hyperchem 8.0.10 [20], and processed for Gaussian input with MolDraw 2.0 [21]. Figures of molecular structures were prepared using Ortep-3 for Windows [22]. For each iron complex, an initial gas phase calculation and wavefunction stability check using the B3LYP/LanL2DZ level of theory was followed by geometry optimization and a frequency calculation, also at the B3LYP/LanL2DZ level, in order to verify the structure as a true minimum and to obtain zero point energies (ZPE's). Finally, single point implicit solvent calculations at the optimized geometries were run, using the B3LYP/6-311+G(d,p) level of theory with SMD solvent corrections and additional wavefunction stability checks. The spin states of the FePPIX complexes were assigned according to the results of our previous study [18], *i.e. S* = 3/2 for the Fe-N (amino) and Fe-O (aqua) complexes and *S* = 5/2 for the Fe-O (alkoxide and hydroxide) complexes. For the free drugs and their zwitterions, a range of different conformations of each molecule was created using Hyperchem, both by systematic searches of torsion angles and by high temperature molecular dynamics runs. The free CQH^+ cation was treated in a similar way, in isolation from the OH^- anion, which was modelled in separate calculations. These input structures were then used for DFT calculations as above, except that the wavefunction stability checks were omitted. All reported energy values include ZPE's, which were scaled by a factor of 0.981 [23].

Results and discussion

As a first step, the energetics of complexation of FePPIX with QN, QD and HN have been modelled by DFT. Although the parasite's digestive vacuole is acidic, individual FePPIX molecules within haemozoin crystals are uncharged overall, and the accretion of a surface layer of like-charged FePPIX-drug complexes on haemozoin should be inherently less favourable than for analogous neutral species. Moreover, the three structurally characterized FePPIX-drug complexes are all neutral [3,4]. Therefore, the calculations were focussed primarily on neutral forms of FePPIX-drug complexes, as the most likely to add to the crystal's surface and prevent further growth. In this study, we have used the same methodology as in our recent work [18]; this was shown to give a good agreement between calculated ΔE values and experimental ΔG values for the binding of a set of six N-donor ligands to the iron(III) site. However, one shortcoming of this approach is its inability to quantify the weak π-π stacking interactions of the drug's aromatic ring systems with the haem of FePPIX. Experiments on substituted porphyrins in aqueous solution gave ΔG values for their π-π interactions with aromatic systems containing 10 and 14 electrons of 3.8 and 4.4 kcal mol^{-1} respectively [24]. QN, QD and CQ all have 10-electron aromatic systems, while HFN has a 14-electron system. Hence, the calculated aqueous complexation energies given in Table 1 are probably underestimated by similar amounts. Experimental studies have shown that such π-stacking interactions are weaker in less polar solvents such as octanol [25].

The X-ray crystal structures of the known complexes were used as the starting point for geometry optimizations. For the free drug molecules, in order to

allow for their conformational flexibility, at least 20 different input geometries were created for each tautomer, and the lowest energy conformer after the solvent calculation was chosen in each case. The results are shown in Table 1. As expected, the energies for zwitterion formation (Scheme 2, step **A**) and reaction with [Fe(PPIXH)] (step **B**) are very similar for QN and QD, which are stereoisomers and form very similar complexes. Formation of the zwitterion is unfavourable, especially in octanol, but the cost is more than recovered by complexation with FePPIX.

<Table 1 about here>

The same procedure was then used to investigate complexation of HN with FePPIX. The X-ray crystal structure of this complex is similar to those of QN and QD, in that the zwitterionic form of the drug uses its alkoxide O to bind to Fe; the main difference is that the protonated amino group hydrogen forms an intermolecular hydrogen bond with a neighbouring FePPIX propionate, rather than the intramolecular hydrogen bonds observed for QN and QD. However, calculations on this system showed that an intramolecular hydrogen bond is also viable for the isolated complex [Fig. 1(a)]. The energies for formation of this complex, by a two-step process analogous to those for QN and QD, are included in Table 1.

Initial calculations on the possible complexation of CQ with FePPIX confirmed that the direct interaction between these species is weak. According to earlier calculations [18], of the three nitrogen atoms in CQ, the secondary amine should offer the strongest coordination to iron. However, geometry optimization of the neutral complex [Fe(PPIX)(CQH)], in which the secondary amine was coordinated to the iron atom in the input geometry, gave a structure with a very long Fe-N distance of 2.598 Å, with the main interaction between the two molecules being a hydrogen

bond between the deprotonated haem propionate group and the protonated tertiary nitrogen of CQH^+. Binding energies for reaction of the neutral species [Fe(PPIXH)] and CQ to give this complex were unfavourable, at +11.6 and +11.2 kcal mol^{-1} in water and octanol respectively. Similarly, geometry optimization of the cationic complex $[Fe(PPIXH)(CQH)]^+$, in which the secondary amine was coordinated to Fe and the protonated tertiary amine of CQH^+ was again hydrogen bonded to a propionate group of PPIXH, gave binding energies of +16.2 and +6.5 kcal mol^{-1} in water and octanol respectively. The calculated Fe-N bond length of 2.323 Å in the optimized geometry is consistent with weak binding of the amine. These results are not unexpected, in the light of experimental solution studies that clearly imply a weak FePPIX-CQ interaction, as mentioned above [13-15]. However, there is another possibility. By direct analogy with QN, QD and HN, CQ could react with water, to give CQH^+ plus hydroxide. Previous calculations on a collection of 30 ligands indicated that hydroxide and alkoxide ligands give the strongest binding to FePPIX [18]. The CQH^+ could then interact with $[Fe(PPIXH)(OH)]^-$ via non-covalent interactions, in particular by hydrogen bonding between its two amino groups and the hydroxide and propionate moieties of the anionic complex, as well as by the same type of π-π stacking interactions observed in the crystal structures of the FePPIX-drug complexes. The energies for formation of the CQH^+/OH^- ion pair, in a process analogous to the zwitterion formation step **A** in Scheme 2, and its subsequent reaction with FePPIX (step **B**) are given in Table 1, and show very similar characteristics to the known zwitterionic systems. The calculated structure of the resulting overall neutral complex is shown in Fig. 1(b).

It should be pointed out that the hypothetical reaction steps **A** and **B** are an aid for analysis of the binding energies, rather than being required as discrete

reaction steps. Thus, further calculations indicated that association of CQH^+ with the neutral complex [Fe(PPIXH)(OH_2)] has an energy of +1.7 kcal mol^{-1} in water and -3.9 kcal mol^{-1} in octanol [Fig. 1 (c)]. As usual, these values neglect the favourable π-π stacking contribution, which should be in the region of 4 kcal mol^{-1} for aqueous solution [24] and rather less than this for a lipid-rich environment [25]. Hence, this form of the complex should be accessible in the environment provided by the parasite's digestive vacuole, and can then be deprotonated to the charge-neutral form most suited to adsorption on the surface of haemozoin crystals.

Conclusion

This work suggests that the molecular basis of the antimalarial action of CQ and related drugs is very similar to that of QN and related drugs. The main difference is that the latter include an alcohol functional group, which can ionize with formation of a zwitterion in order to give a strong Fe-O (alkoxide) bond, whereas the former rely on water to provide a strongly bound hydroxyl ligand, which is then stabilized by non-covalent interactions with the drug. Since the pK_a of the aqua ligand when bound to FePPIX is ~7.3 [26,27], in the absence of the drug a strongly bound hydroxyl ligand will rapidly protonate in the acidic environment of the parasite's digestive vacuole to give a weakly bound aqua ligand, allowing haemozoin crystallization to proceed as required by the parasite. It appears that CQ and related drugs can stabilize the iron(III) hydroxyl complex, poisoning the fast growing surfaces with a monolayer of the [Fe(OH)(PPIXH)(CQH)] complex. Our ongoing calculations suggest that the same model is applicable to other CQ-type drugs such as amodiaquine, primaquine and mepacrine. With respect to the search for new antimalarial drugs, the possibility

of interaction of candidate molecules with the hydroxyl-bound FePPIX, as well as with FePPIX itself, needs to be considered.

Acknowledgements

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Footnotes

† Throughout this paper, FePPIX is used to indicate the generic form of ferriprotoporphyrin IX. Specific neutral and ionized forms of the protoporphyrin IX ligand are denoted as $PPIXH_2$, $PPIXH^-$ and $PPIX^{2-}$.

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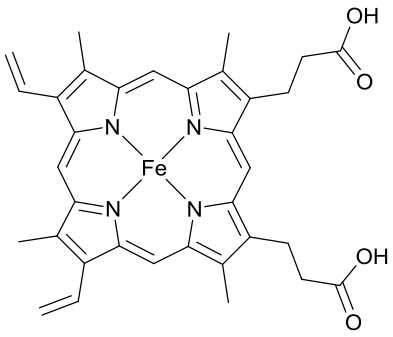
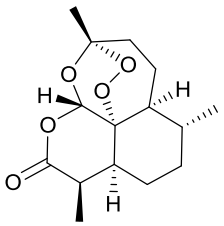
Table 1 Calculated energies for reactions of QN, QD, HN and CQ with FePPIX in water (values in *n*-octanol are given in parentheses).[a]

Step	QN ΔE, kcal mol^{-1}	QD ΔE, kcal mol^{-1}	HN ΔE, kcal mol^{-1}	CQ ΔE, kcal mol^{-1}
A	+13.8 (+18.6)	+13.4 (+18.5)	+19.2 (+26.3)	+15.2 (+30.7)
B	-18.9 (-31.5)	-19.5 (-31.8)	-19.8 (-32.2)	-15.3 (-38.2)
A+**B**	-5.1 (-12.9)	-6.1 (-13.3)	-0.6 (-5.8)	0.0 (-7.5)

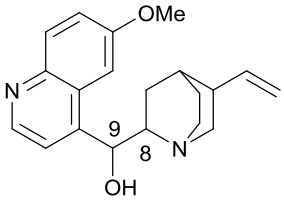
[a] Obtained at the B3LYP/6-311+G(d,p) level of theory, and including ZPE's. Step **A** refers to the formation of the zwitterion of the free drug for QN, QD and HN, or the CQH^+/OH^- ion pair for CQ; step **B** refers to coordination of these charged species to the iron(III) centre of the neutral complex [Fe(PPIXH)], see Scheme 2.



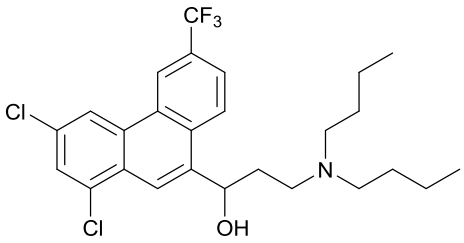
Iron ferriprotoporphyrin IX (FePPIX)



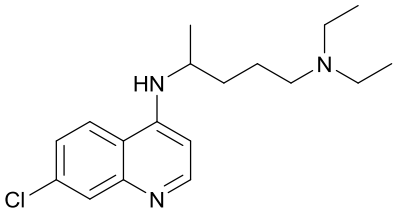
Artemisinin (AM)



Quinine (QN) and Quinidine (QD)

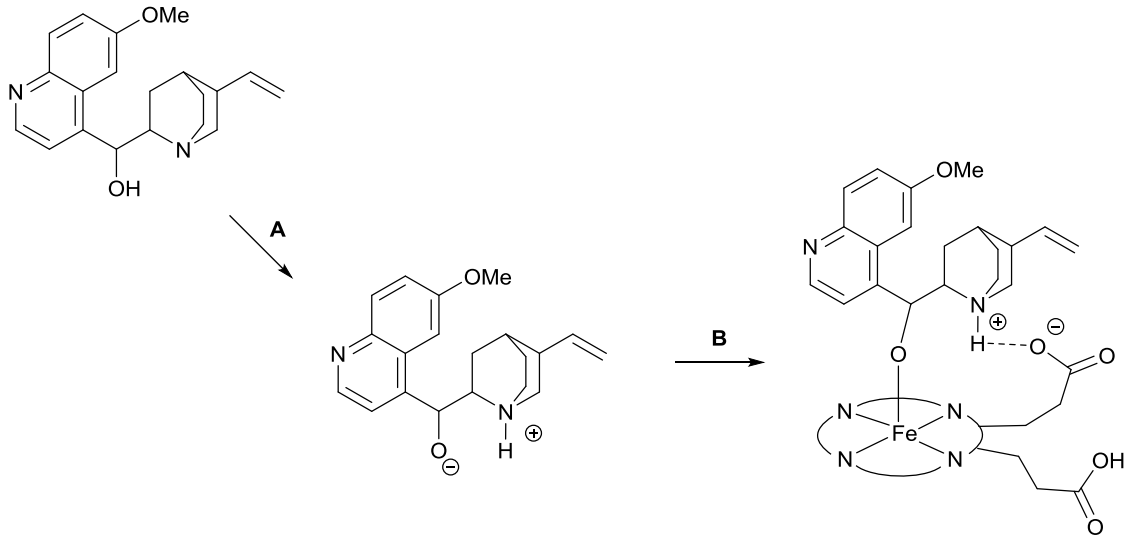


Halofantrine (HN)



Chloroquine (CQ)

Scheme 1. Structures of iron ferriprotoporphyrin IX and some important antimalarial drugs that target this species. Quinine is the (8*S*, 9*R*) stereoisomer and quinidine is the (8*R*, 9*S*) stereoisomer.



Scheme 2. Formation of the zwitterionic forms of QN and QD (step **A**), followed by coordination to [Fe^{III}(PPIXH)] to give an overall neutral complex (step **B**).

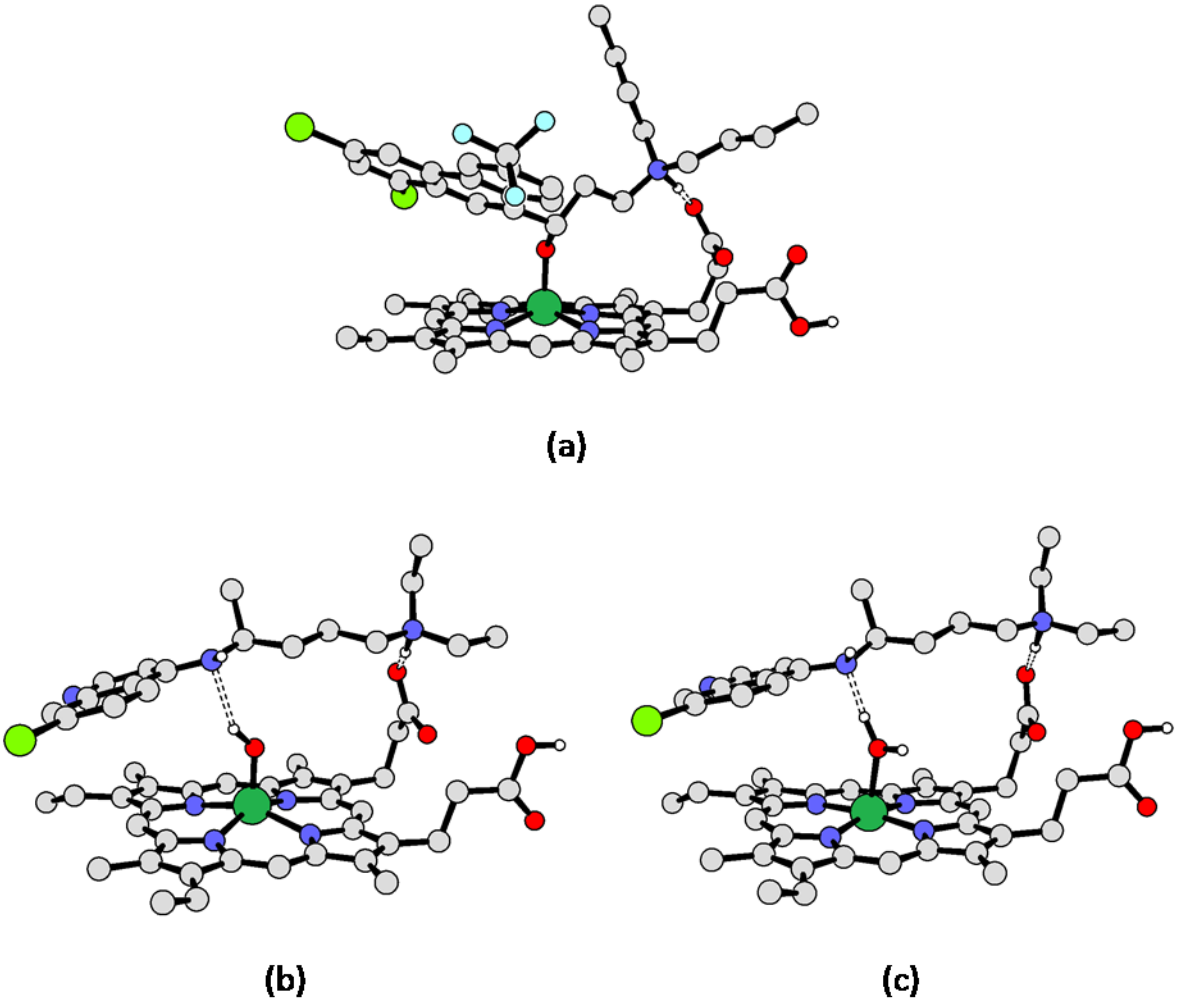


Fig. 1 Calculated structures of (a) the neutral [Fe^{III}(PPIXH)(HN)] complex, incorporating an intramolecular hydrogen bond between the propionate -CO_2^- group and tertiary NH^+ of the halofantrine zwitterion, (b) the neutral [Fe^{III}(PPIXH)(OH)(CQH)] complex, and (c) the cationic [Fe^{III}(PPIXH)(H_2O)(CQH)]$^+$ complex. Hydrogen bonds are shown as dashed lines. Atom colouring: carbon, grey; chlorine, light green; fluorine, cyan; hydrogen, white; iron, dark green; oxygen, red; nitrogen, blue. Hydrogen atoms attached to carbon are omitted for clarity.