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Solution structures of chloroquine–ferriheme complexes modeled using MD simulation and investigated by EXAFS spectroscopy

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ABSTRACT

The interaction of chloroquine (CQ) and the μ-oxo dimer of iron(III) protoporphyrin IX (ferriheme) in aqueous solution was modeled using molecular dynamics (MD) simulations. Two models of the CQ-(μ-oxo ferriheme) complex were investigated, one involving CQ π-stacked with an unligated porphyrin face of μ-oxo ferriheme and the other in which CQ was docked between the two porphyrin rings. The feasibility of both models was tested by fitting computed structures to the experimental extended X-ray absorption fine structure (EXAFS) spectrum of the CQ-(μ-oxo ferriheme) complex in frozen aqueous solution. The docked model produced better agreement with experimental data, suggesting that this is the more likely structure in aqueous solution. The EXAFS fit indicated a longer than expected Fe–O bond of 1.87 Å, accounting for the higher than expected magnetic moment of the complex. As a consequence, the asymmetric Fe–O–Fe stretch shifts much lower in frequency and was identified in the precipitated solid at 744 cm^{-1} with the aid of the O^{18} isomer shift. Three important CQ-ferriheme interactions were identified in the docked structure. These were a hydrogen bond between the oxide bridge of μ-oxo ferriheme and the protonated quinolinium nitrogen atom of CQ; π-stacking between the quinoline ring of CQ and the porphyrin rings; and a close contact between the 7-chloro substituent of CQ and the porphyrin methyl hydrogen atoms. These interactions can be used to rationalize previously observed structure–activity relationships for quinoline–ferriheme association.

1. Introduction

The solution structure of the complex of the antimalarial chloroquine (CQ) and iron(III) protoporphyrin IX (ferriheme) has been a topic of investigation for many years [1,2]. This interest has primarily stemmed from proposals that CQ inhibits the formation of hemozoin, a less toxic crystalline product formed in a crucial ferriheme detoxification process within the malaria parasite [3–6]. In early studies CQ was proposed to inhibit hemozoin formation through complexation with free ferriheme in aqueous solution, thus preventing its incorporation into the growing hemozoin crystal [4,7]. Later, it was proposed that CQ caps the growing hemozoin crystal [8,9]. More recently it has been hypothesized that CQ inhibits hemozoin formation by docking onto the fastest growing crystal face [10]. This idea has been gaining favor based on the findings of several investigations [6,11–13], and is now generally considered the more likely mechanism of inhibition. Nonetheless, the CQ–ferriheme complex remains of interest. This is because the resultant free ferriheme arising from hemozoin inhibition would likely still complex with excess CQ present in the malaria parasite. Thus, the CQ–ferriheme complex may well influence the subsequent toxic effects of ferriheme on the parasite, ultimately leading to its death.

Several studies have previously suggested possible structures of the CQ–ferriheme complex [14–24], however, many of these are contradictory. Examples of some of these models (see Fig. 1) include CQ coordinated through its quinoline nitrogen atom to the iron center of monomeric ferriheme (**1**) [17]; a CQ molecule π-stacking with the outer unligated porphyrin face of a μ-oxo ferriheme dimer (**2**) [16,20]; two CQ molecules π-stacking with the outer faces of a ferriheme tetramer adduct where two μ-oxo ferriheme dimers are reciprocally coordinated via their propionate side chains (**3**) [21]; and a CQ molecule π-stacking between the unligated faces of μ-oxo dimeric ferriheme aggregates (**4**) [14,22]. Consequently, there is no consensus regarding the structure of the CQ–ferriheme complex in solution. The confusion surrounding this topic has in some cases arisen from incomplete knowledge of the effect of experimental conditions on ferriheme speciation. This lack of information has sometimes been exacerbated by use of either single experimental or computational methods to propose structural models of the complex, often without further validation using other independent techniques. Indeed, careful consideration of these structures reveals that **1** and **5** are inconsistent with the observed

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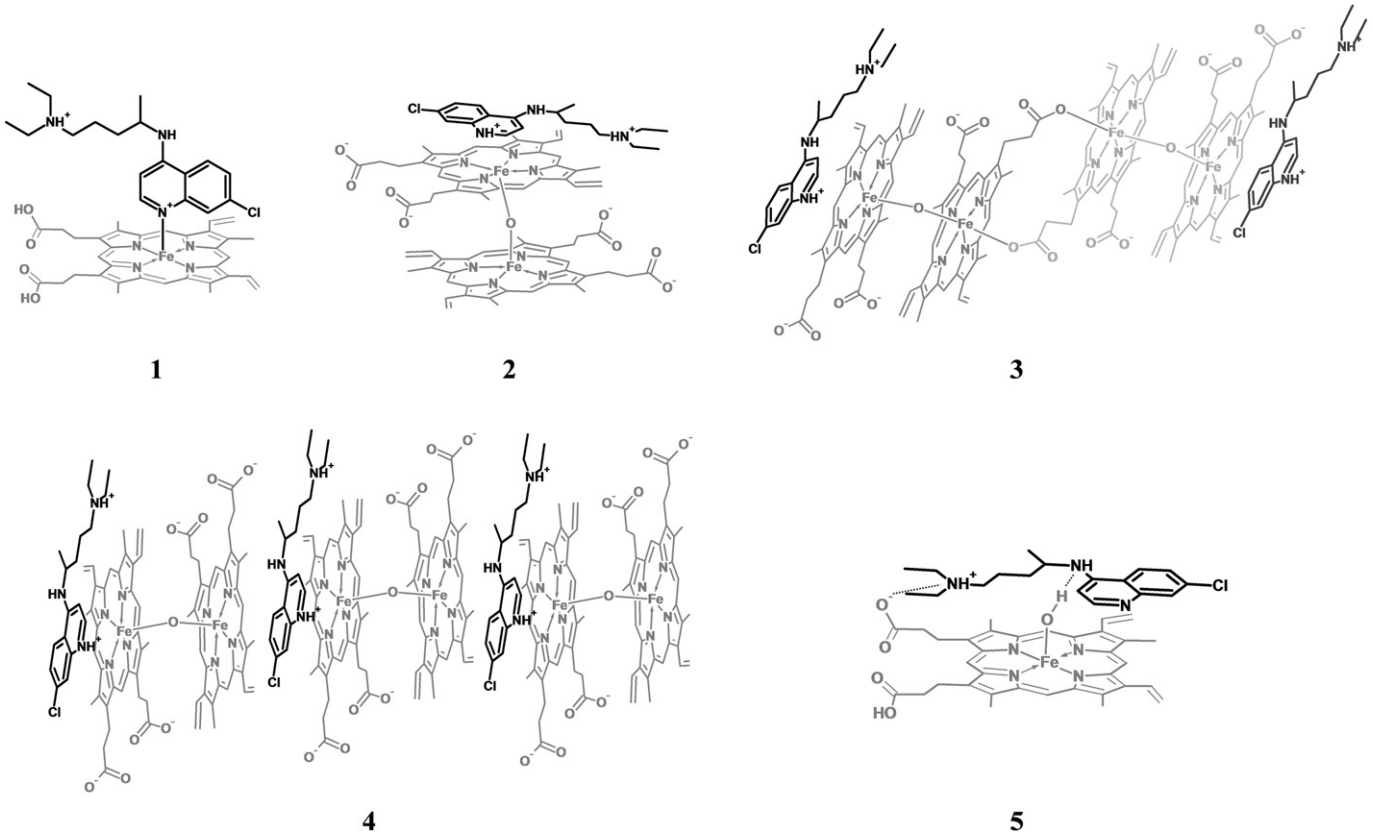

Fig. 1. Examples of structures previously proposed for the CQ–ferriheme complex. See text for description of the structures and their relevant references.

stoichiometry of the complex, while **3** would be expected to give rise to characteristic strong infrared bands around 1660 and 1210 cm^{-1} that are not observed.

In a recent computational study using quantum mechanical calculations, Acharige and Durant showed that a structure in which CQ hydrogen bonded to a propionate side chain and axial hydroxide or water ligand of ferriheme through its tertiary amine and 4-amino substituent groups respectively (**5**) was stable in an implicit octanol model [23]. Interestingly, the structure was far less stable in implicit water, although the authors ascribed this to the inability of the method to properly account for π-stacking interactions. These findings, combined with experimental evidence that ferriheme is present as a μ-oxo dimer in the presence of CQ in a 2:1 ratio in aqueous solution [25] indicate that this newly proposed structure may be relevant to lipid, but not aqueous environments within the cell.

Even more recently, the first example of a crystal structure of a porphyrin complex of CQ, that with Ga(III) protoporphyrin IX, was reported [24]. In this structure obtained from methanol solution, Ga(III) protoporphyrin IX was 6-coordinate and existed as a μ-propionato dimer with CQ hydrogen bonded to a methoxide ligand and the coordinated propionate side chain via the quinoline N atom and tertiary amino group respectively. The authors suggested that a similar structure may exist with ferriheme in aqueous solution in which the Fe(III) ion is in a low spin state with water as the sixth ligand. Previous studies using extended X-ray absorption fine structure (EXAFS) spectroscopy provided evidence that CQ interacts with the μ-propionato dimer of Fe(III) mesoporphyrin IX in DMSO solution [26], while in acetic acid and aqueous acetic acid solution this dimer apparently dissociated and no direct evidence for interaction with CQ could be discerned within 4 Å of the iron center [27]. Thus, while these structures provide insight into interactions with hemozoin that may be pertinent to the mechanism of its inhibition, existence of a CQ-(μ-propionato ferriheme) complex as the dominant species in aqueous solution seems far less certain. Furthermore, low spin ferriheme complexes have characteristic UV–visible absorbance and magnetic circular dichroism spectroscopic features that are absent in the spectrum of the CQ–ferriheme complex in aqueous solution [25]. In addition, the characteristic infrared bands of the coordinated propionate groups at 1660 and 1210 cm^{-1} are not observed in the precipitate [25] and the Mössbauer spectrum of the complex is consistent with the μ-oxo ferriheme dimer [28].

In a recent experimental investigation of the interaction of CQ and ferriheme in aqueous solution using multiple techniques we showed that CQ bound ferriheme in the μ-oxo dimeric form in a 1:2 CQ:ferriheme molar ratio [25]. In view of the observation that CQ causes a redistribution of heme from the acidic digestive vacuole of the malaria parasite into the cytoplasm [6], it would seem likely that any such complex present in the parasite exists in this form. We have now undertaken a computational and experimental investigation of its structure. We have probed two possible conformations of the complex using molecular dynamics (MD) simulations. The aim of this investigation was to use a newly parameterized force field that we have recently developed specifically for five-coordinate non-protein bound ferriheme species [29] to produce an improved aqueous solution CQ–ferriheme model. To support this model, we investigated its ability to fit the experimental EXAFS spectrum of the complex in frozen aqueous solution. A new structure proposed for the CQ–ferriheme complex could successfully fit the EXAFS spectrum, was consistent with magnetic and infrared spectroscopic data and could rationalize previously reported trends in quinoline–ferriheme association constant data.

2. Experimental and computational methods

2.1. EXAFS data collection and refinement

With the exception of hemin (Fluka), all chemicals were purchased from Sigma-Aldrich. The EXAFS spectrum of the CQ-(μ-oxo ferriheme)