Drug Interactions

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Soluble Synthetic Analogues of Malaria Pigment: Structure of Mesohematin Anhydride and its Interaction with Chloroquine in Solution**

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The rise of resistance to the quinoline and trioxane antimalarial drugs[1] adds urgency to the search for new therapies for treating this pernicious protozoan. It is significant that both quinine and artemisin are amongst the oldest drugs with ethnopharmacological origins,[2] and that considerable medicinal chemistry effort has led to closely related derivatives such as chloroquine, mefloquine, and arterolane. Given the difficulty in developing vaccines for any of the life stages of plasmodia,[3] new strategies, targets, and drugs must be found in the near future.[4] Although considerable uncertainty still lingers about the specific details of the drug targets of the quinoline and trioxane antimalarial drugs,[5] consensus has emerged that the quinoline antimalarial drugs interfere with normal hemoglobin processing in the digestive vacuoles of the red blood cells of plasmodia.[5c] One indication of the pressing need to understand the details of this pathway is the recent confirmation that the structure of the native malaria pigment hemozoin (Hz) is identical to that of synthetic hematin anhydride (β-hematin).[6] This result nicely confirms the prior results based on spectroscopy[7] and powder diffraction.[8] Although differences in the mosaicity (the long-range order of a crystal) and morphology of the natural and synthetic materials may exist, and these will remain problematic for X-ray diffraction studies, the redetermination led to the hypothesis that π stacking of the heme rings is critical in the formation of hematin anhydride.[6] Herein we report the structure of a closely related isostructural synthetic dimer based on mesoporphyrin. Similar to the naturally occurring malaria pigment hematin anhydride, mesohematin anhydride (**1**) consists of propionate-bound dimers. Among the important consequences of the substitution of ethyl or hydrogen for vinyl groups is an improved solubility of **1** and **2**, which allows for the first direct spectrophotometeric titrations of these dimers with chloroquine.

Mesohematin anhydride, [{Fe(MP-IX)}$_2$] (**1**), and deuterohematin anhydride, [{Fe(DP-IX)}$_2$] (**2**), are readily prepared by treating dry solutions of their halides with the noncoordinating base 2,6-lutidine (Scheme 1). Similar synthetic conditions as for the dehydrohalogentation can also be used to prepare synthetic hematin anhydride.[8c] As found for hematin anhydride, both **1** and **2** are air-stable, black, water-insoluble, microcrystalline solids with strong bands in their IR spectra consistent with η^1-propionate–iron coordination[9] and with the formation of a propionic acid dimer.[7b,10] Unlike hematin anhydride, **1** and **2** are slightly soluble in dichloromethane, propionic acid, and acetic acid. For example, the spectrum of **1** in dichloromethane shows a broad Soret band at 378 nm and Q bands at 502, 533, and 631 nm. Diffuse reflectance spectra of the isolated solid dispersed in finely ground potassium bromide show bands with similar intensities at these energies. The spectra of **1**, both in solution and in the solid state, show bands consistent with a five-coordinate high-spin iron(III) center and with no evidence for a strong π-stacking interaction, such as that seen in the J-aggregates of the anionic sulfonated synthetic porphyrins.[11] Moreover, recent EXAFS data on solutions of **1** and **2** have been interpreted as being due to the dimer, which suggests that in the absence of a strong base or strong coordinating bases the dimer structures of **1** and **2** are retained in solution.[12] Both acetic and propionic acid dissolve **1** and **2** to give even more concentrated solutions, and EXAFS studies demonstrate that the dimer structure is retained until the acid is diluted with additional solvent.[13]

Microcrystals of **1** form under the conditions shown in Scheme 1, and these give excellent X-ray powder diffraction data (see Figure S1 in the Supporting Information), which has allowed for characterization of the structure of mesohematin anhydride in solution (Figure 1).[14] A surprising and significant difference between the structure of **1** and hematin anhydride is the presence of a dimethylsulfoxide solvate, which is hydrogen bonded to the free propionic acid side chain. Although this changes the crystal packing arising of the free propionic acid side chain, the geometry of the five-

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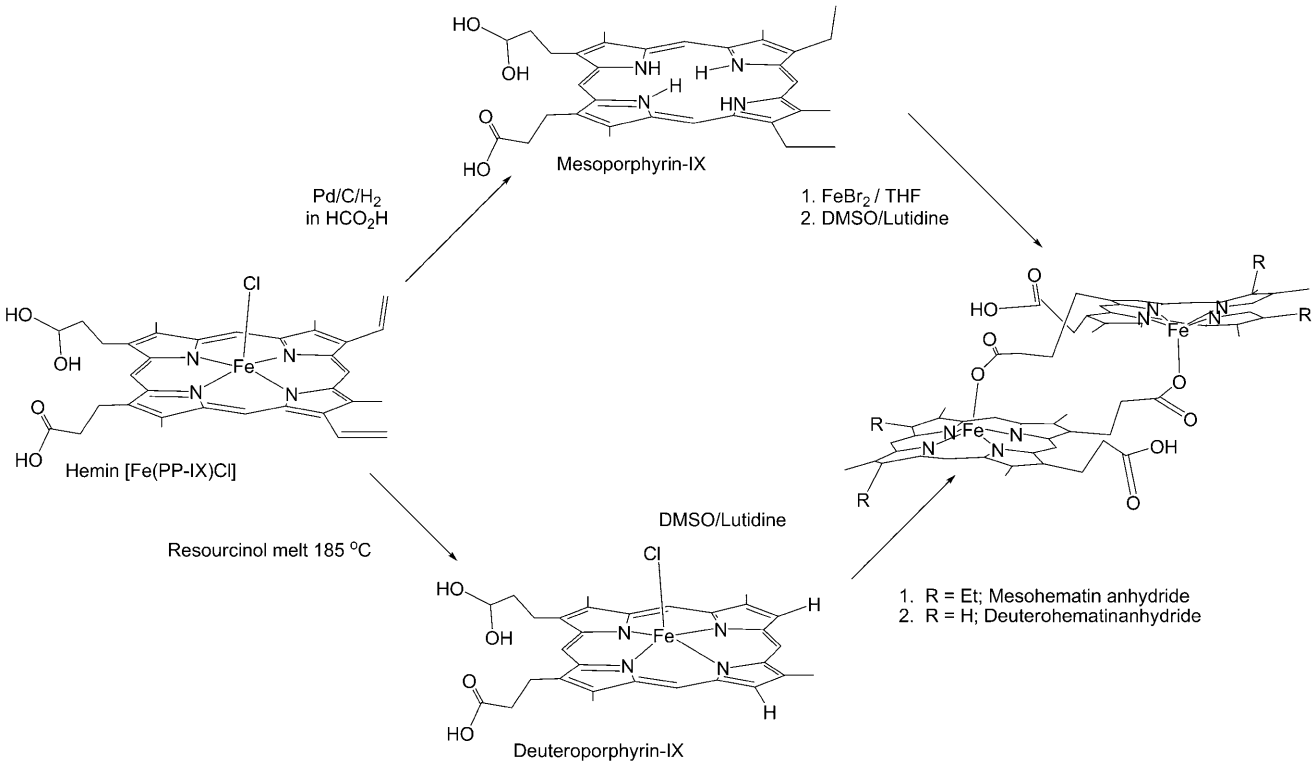
Supporting information for this article (crystallographic details for the structure of **1** as well as additional diagrams depicting the bonding and stacking in this structure, spectra, and images of the solutions of the new malaria pigment analogues) is available on the WWW under http://dx.doi.org/10.1002/anie.201100910.



Scheme 1. Synthesis of hematin anhydride analogues.

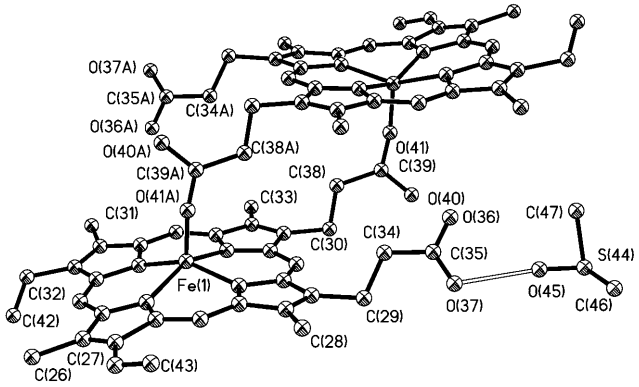


Figure 1. View of the mesohematin anhydride dimer in **1** showing hydrogen bonding between O(37) and O(45) from the propionic acid side chain to the DMSO solvate.

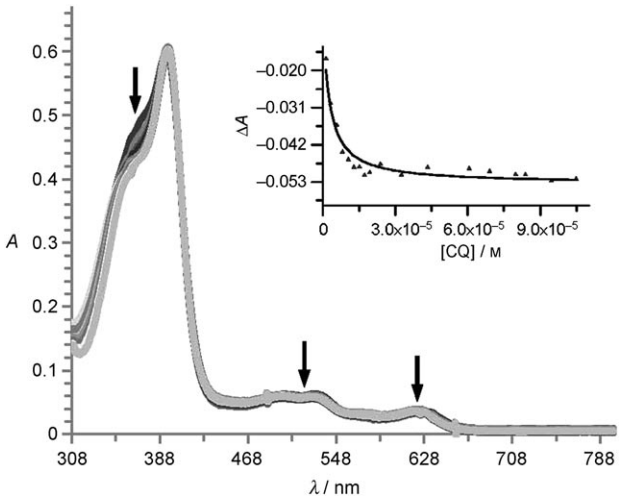


Figure 2. Change in the UV/Vis spectrum on addition of a solution of 0–1.08×10^{-4} M CQ in CH_2Cl_2 to a 2.92×10^{-6} M solution of **2** in CH_2Cl_2 at 296 K. The inset shows the plot of the nonlinear fit of the data at 367 nm.

coordinate high-spin iron center and the core geometry of the porphyrin remains similar to that in hematin anhydride.

Substitution of the vinyl groups in hematin anhydride with either ethyl groups in **1** or by protons in **2** results in a modest (see Figure S3 in the Supporting Information), but useful, solubility in organic solvents. These solvents mimic the hydrophobic environment in the lipid nanodrops which are proposed to mediate the synthesis of hemozoin in the parasite's digestive vacuole.[15] One remarkable feature of hematin anhydride is its profound insolubility. Among the critical experiments permitted by the improved solubility of **1** and **2** are spectrophotometric titrations of the dimers with chloroquine. As depicted in Figure 2, treating a solution of **2** in dichloromethane with chloroquine (CQ) causes a moderate decline in the intensity of the trace in the Soret band region at about 305–390 nm and a slight red shift of the absorbance maxima (ca. 1 nm). All the described changes become evident with the first addition of the aliquot (1 equivalent of chloroquine) and ceases when at least 37 equivalents of chloroquine are added, during which the Q bands remain

unaltered in energy or intensity and the titration can be fit to a single-step weak binding with $\log K_{11} = 5.6$ for **2**.

Inhibition of hemozoin formation by antimalarial drugs is believed to occur through the high affinity they have for one or more of the various possible chemical forms of monomeric or dimeric [Fe^{III}(PP-IX)] that are likely to be found in the digestive vacuole of the parasite.[16] There are numerous studies reporting the interaction between CQ and natural and synthetic monomeric or μ-oxo dimeric porphyrin species,[17] and most of them describe this interaction as occurring in a π–π fashion between the aromatic system of the porphyrin and the quinoline ring in CQ. Most importantly, the coordination of an electron-rich center of CQ to any of the metallic iron(III) centers in **2** would be unlikely if each Fe^{III} center is moved out of the plane of the porphyrin ring toward the propionate group of the other porphyrin, as occurs with **1**, **2**, and hematin anhydride. The change in the UV/Vis spectrum during the titration supports two important points: First, at low concentration there is minimal self-aggregation of **2**, as evident by no broadening of the Soret band. Second, there is direct evidence in favor of a noncovalent bonding between CQ and **2**, since coordination by any of the chloroquine nitrogen atoms is expected to change the nature of the Q bands.[18] The formation of a π–π complex between **2** and CQ would lead to the observed hypochromism in the Soret band.[19,20]

In conclusion, we have prepared new isostructural synthetic malaria pigment mimics with useful solubility in organic solvents. We have determined the weak binding constants of chloroquine to these dimers under these conditions, and the findings suggest that solution-phase heme/drug interactions alone are unlikely to be the origin of action of the chlorquine drug. A model in which the drug binds to the growing hemozoin surface remains an important target.

Experimental Section[21]

[{Fe(MP-IX)}$_2$] (**1**): [Fe(MP-IX)Cl] (109 mg, 0.167 mmol) was dissolved in a mixture of methanol (14 mL) and DMSO (7 mL). 2,6-Lutidine (2 mL, 17 mmol) was added and the mixture was agitated by stirring (10 min) and stored under an inert atmosphere. After 21 days, the solvent was decanted off and the resultant crystalline precipitate was dried in a vacuum oven for 48 h at room temperature to give the crude solid. The solid was washed (×2) with methanol (2 h), centrifuged (5000 rpm, 1 h, RT) and separated from the liquid by decanting. Yield = 69 mg, 67%. M.p. 182 °C (decomp); IR (KBr): $\tilde{\nu} = 1719, 1656, 1208, 1146\ cm^{-1}$; UV/Vis($CH_2Cl_2$): λ_{max} ($\log\varepsilon$; $M^{-1}cm^{-1}$): Soret band: 378 nm (5.11); Q bands: 502 (4.12), 533 (4.60), 631 (3.96) nm. Elemental anal. calcd for $C_{68}H_{70}N_8O_8Fe_2 \cdot C_2H_6SO$: C 63.92, H 5.78, N 8.52; found: C 64.15, H 5.80, N 8.61.

[{Fe(DP-IX)}$_2$] (**2**): [Fe(DP-IX)Cl][22] (100 mg, 0.17 mmol) was dissolved in a mixture of methanol (14 mL) and DMSO (7 mL). 2,6-Lutidine (2 mL, 17.22 mmol) was added and the mixture agitated until homogeneous and then stored dry under an inert atmosphere or undisturbed over P_2O_5. After 20–25 days, the solvent was decanted off and the resultant crystalline precipitate was dried in vacuo for 48 h at room temperature. Yield = 62 mg, 65%. Additional purification: washing (50 mL) with $NaHCO_3$ solution (0.01M, 3 h; ×2), water (1 h), and methanol (1 h). Between washes the suspension was centrifuged (3000 rpm, 3 h, RT), before finally drying under vacuum. M.p. 283 °C (decomp); IR (KBr): $\tilde{\nu} = 1732, 1654, 1208\ cm^{-1}$. UV/Vis($CH_2Cl_2$) λ_{max} ($\log\varepsilon$; $M^{-1}cm^{-1}$): Soret band: 394 nm (5.28); Q bands: 500 (4.23), 528 (4.13), 620 (4.20) nm. Elemental anal. calcd for $C_{60}H_{54}N_8O_8Fe_2$: C 63.96, H 4.83, N 9.94%; found: C 63.49, H 4.15, N 9.40%.

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