A Novel Endogenous Antimalarial: Fe(II)-Protoporphyrin IXα (Heme) Inhibits Hematin Polymerization to β-Hematin (Malaria Pigment) and Kills Malaria Parasites†

Diego Monti,‡ Bruno Vodopivec,§ Nicoletta Basilico,|| Piero Olliaro,⊥ and Donatella Taramelli*,||

Dipartimento di Chimica Organica e Industriale, CSSON, CNR, Dipartimento di Chimica Fisica ed Elettrochimica, Istituto di Microbiologia, Università di Milano, Milano, Italy, and UNDP/WorldBank/WHO Special Program for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland

Received January 13, 1999; Revised Manuscript Received March 25, 1999

ABSTRACT: The polymerization of hemoglobin-derived ferric-protoporphyrin IX [Fe(III)PPIX] to inert hemozoin (malaria pigment) is a crucial and unique process for intraerythrocytic plasmodia to prevent heme toxicity and thus a good target for new antimalarials. Quinoline drugs, i.e., chloroquine, and non-iron porphyrins have been shown to block polymerization by forming electronic π−π interactions with heme monomers. Here, we report the identification of ferrous-protoporphyrin IX [Fe(II)PPIX] as a novel endogenous anti-malarial. Fe(II)PPIX molecules, released from the proteolysis of hemoglobin, are first oxidized and then polymerized to hemozoin. We obtained Fe(II)PPIX on preparative scale by electrochemical reduction of Fe(III)PPIX, and the reaction was monitored by cyclic voltammetry. Polymerization assays at acidic pH were conducted with the resulting Fe(II)PPIX using a spectrophotometric microassay of heme polymerization adapted to anaerobic conditions and the products characterized by infrared spectroscopy. Fe(II)PPIX (a) did not polymerize and (b) produced a dose-dependent inhibition of Fe(III)PPIX polymerization (IC_{50} = 0.4 molar equiv). Moreover, Fe(II)PPIX produced by chemical reduction with thiol-containing compounds gave similar results: a dose-dependent inhibition of heme polymerization was observed using either L-cysteine, *N*-acetylcysteine, or DL-homocysteine, but not with L-cystine. Cyclic voltammetry confirmed that the inhibition of heme polymerization was due to the Fe(II)PPIX molecules generated by the thiol-mediated reduction of Fe(III)PPIX. These results point to Fe(II)PPIX as a potential endogenous antimalarial and to Fe(III)PPIX reduction as a potential new pharmacological target.

Plasmodia digest hemoglobin during their intraerythrocytic life cycle in a specialized organelle (the digestive or food vacuole, analogous to phagolysosomes of mammalian cells) through the combined action of at least two aspartic proteinases (plasmepsins I and II) and one cysteine proteinase (falcipain) *(1−3)*. Four molecules of heme [ferrous-protoporphyrin IX, Fe(II)PPIX],[1] the prosthetic group of hemoglobin, are released from each molecule of hemoglobin when digested by the malaria parasite. It is commonly accepted that, in the presence of molecular oxygen in the parasite food vacuole, Fe(II)PPIX is oxidized to form Fe(III)PPIX following a Fenton−Haber−Weiss-type reaction: at that time, a cascade of events begins, on one hand, to scavenge the reactive oxygen species produced and, on the other hand, to polymerize Fe(III)PPIX to β-hematin (hemozoin or malaria pigment) *(3−5)*. The polymer forms via the coordination of the central iron atom of one molecule to one carboxyl of the adjacent molecule *(6)*. Whether the reaction in the parasite food vacuole occurs spontaneously or a scaffolding molecule, such as histidine rich protein II, or a catalyst is needed is still an open question *(7−9)*. Synthetic β-hematin obtained chemically in vitro in acidic conditions retains the solubility and spectroscopic characteristics of the native pigment *(6, 10−12)*.

Polymerization to β-hematin appears to prevent heme toxicity *(1, 6)* and is also thought to be the primary target of the quinoline-type antimalarial drugs *(10, 13)*. Chloroquine seems to act by forming complexes with hematin, which terminate hemozoin chain extension *(14, 15)*. NMR, UV−visible, and Mossbauer spectroscopic data indicate that π−π interactions between the drug and the electronic system of hematin govern the formation of these adducts *(16, 17)*. Consequently, it was hypothesized that any molecule able

†This work was supported in part by UNDP/World Bank/WHO Special Program for Research and Training on Tropical Diseases, Geneva, (CH) N. 97-0115/98-0363, and by the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Progetti di Rilevante Interesse Nazionale, Cofinanziamento 1997.

* To whom correspondence should be addressed at the Istituto di Microbiologia, Via Pascal 36, 20133 Milano, Italy. E-mail: Donatella.Taramelli@unimi.it.

‡ Dipartimento di Chimica Organica e Industriale, Università di Milano.

§ Dipartimento di Chimica Fisica ed Elettrochimica, Università di Milano.

|| Istituto di Microbiologia, Università di Milano.

⊥ UNDP/WorldBank/WHO Special Program for Research and Training in Tropical Diseases (TDR).

[1] Abbreviations: Fe(II)PPIX, ferrous-protoporphyrin IX; Fe(III)PPIX, ferric-protoporphyrin IX; NAC, *N*-acetylcysteine; 2-ME, 2-mercaptoethanol; FT-IR, Fourier transform infrared spectroscopy; HPIA, heme polymerization inhibitory activity.

10.1021/bi990085k CCC: $18.00
Published on Web 06/19/1999

to form $\pi-\pi$ interaction with hematin could inhibit the polymerization to β-hematin. This hypothesis has been confirmed by a previous observation of ours that porphyrins lacking a metal center not only cannot polymerize but also inhibit heme polymerization *(12)*.

Based on these observations, we set out to assess the role of the oxidative status of the central iron of the heme molecule in the polymerization process. (i) Fe(II)PPIX is the moiety released in the food vacuole during hemoglobin digestion; (ii) its electronic structure supports the formation of $\pi-\pi$ interactions; (iii) the reduced state of iron does not support the formation of the iron−carboxylate bonds between heme subunits to form β-hematin.

To assess the effects of Fe(II)PPIX on hematin polymerization, Fe(II)PPIX had to be prepared from Fe(III)PPIX in sufficient amounts under controlled conditions. First, electrochemical reduction was used to characterize the reaction products in the absence of chemical interference with the polymerization assay. Subsequently, reducing agents (electron donors), shown to inhibit hematin polymerization *(9)*, were employed: thiol-containing molecules were selected on the basis of either their pharmacological or their physiological antioxidant properties.

EXPERIMENTAL PROCEDURES

All the chemical reagents used in our assays, unless specified, were obtained from Sigma/Aldrich, Milan, Italy.

Reduction of Fe(III)PPIX and Cyclic Voltammetry. (A) Electrochemical Reduction. A solution of Fe(III)PPIX was prepared by dissolving hemin [Fe(III)protoporphyrin IX chloride] in a small volume of KOH and diluting to 5 mM concentration with a solution of potassium bicarbonate (5 mM) in deionized distilled water. This solution was degassed with several cycles of pumping under N_2 and immediately used in the preparative electrolysis or in the microassay for heme polymerization *(18)*. The solution of hemin was electrolyzed in a preparative cell divided by a membrane. The anodic compartment contained a solution of 1 M KCl and the cathodic compartment 50 mL of 5 mM solution of hematin. The working electrode was a Pt plate (25 × 50 mm). The solution was maintained under nitrogen with stirring at a constant potential of −0.55 to −0.6 V, using the Amel 5000 electrochemical apparatus equipped with a micro Pt electrode integrated in the preparative cell, thus allowing in situ cyclic voltammetric measurements. The analytical voltammograms were consistent with previous reports *(19−21)*.

(B) Chemical Reduction. A solution of 40 μmol of hemin dissolved in 10 mL of 0.1 M NaOH was prepared and kept under nitrogen with stirring. Then, 40 μmol of cysteine or *N*-acetylcysteine (NAC) (Zambon Group, Milan, Italy) was added in 5 mL of glacial acetic acid. The incubation was prolonged for 8 h at 37 °C. At the end, 7 mL of 10 M NaOH was added to solubilize β-hematin and the clear solution used for the following steps. For analytical cyclic voltammetry, 15 mL of EtOH and 2 g of $NaNO_3$ were added to the above solutions which were then analyzed in the electrochemical undivided cell. Linear cyclic voltammetry was done with a Pt electrode (calomel reference electrode) at a scan rate of 40 mV/s from −300 to −50 mV. The chemical reoxidation of Fe(II)PPIX was obtained with a stream of air through the cell for a few minutes, and the solution was analyzed under the same conditions reported above.

In Vitro Hematin Polymerization. β-Hematin was synthesized in accordance with the procedure reported by Egan et al. *(10)* with slight modifications. Briefly, a solution of 5 mM hematin (ferriprotoporphyrin IX hydroxide) was prepared as described above. Then a solution containing 0.3 mL of 1 M HCl plus 1.74 mL of 12.9 M sodium acetate (pH 5.0), prewarmed at 60 °C, was added. After 60 min incubation at 60 °C, the reaction mixture was filtered over a type HA Millipore filter (0.45 μm) and washed extensively with distilled water. The solid precipitate was then dried over phosphorus pentoxide under vacuum overnight and finally characterized by Fourier transform infrared (FT-IR) spectroscopy using a Jasco FT-IR spectrometer. The iron−carboxylate bonds present in the synthetic polymer were visualized by FT-IR spectroscopy as two characteristic bands at 1662 and 1209 cm^{-1}.

Microassay for Hematin Polymerization. To evaluate the heme inhibitory activity of Fe(II)PPIX and of reducing agents, we used the spectrophotometric microassay of heme polymerization recently developed in our lab *(18)*. Slight technical modifications were introduced to adapt the microassay to anaerobic conditions: a small polyethylene atmosbag, with two inlet accesses per side, sealed and inflated with inert gas was utilized. The experiments were conducted under continuous nitrogen flux. One hundred microliter solutions of Fe(II)/Fe(III)PPIX in different ratios were distributed in quadruplicate in 96-well U-bottom microplates (0.5 μmol of total porphyrin/well) (Costar 3799). Hematin was precipitated by the addition of 0.8 mmol of acetic acid (50 μL) and the plates were incubated for 24 h under nitrogen at room temperature. At the end of the incubation, the plates were taken out of the atmosbag and centrifuged at 3300*g* for 15 min, and the soluble fraction (fraction I = unprecipitated haematin) of unreacted material was collected. The remaining pellets were resuspended with 200 μL of DMSO to remove unpolymerized hematin. Plates were then centrifuged again at 3300*g* for 15 min. The DMSO-soluble fraction (fraction II = unpolymerized hematin) was collected, and the pellets, consisting of a pure precipitate of β-hematin, were dissolved in 0.1 M NaOH (fraction III = solubilized β-hematin). A 150 μL aliquot of each fraction (I−II−III) was transferred to a new plate, and serial 4-fold dilutions in 0.1 M NaOH were made. The amount of hematin was determined by measuring the absorbance at 405 nm using a microtiter plate reader (Molecular Devices). A standard curve of hematin dissolved in 0.1 M NaOH was used to calculate the amount of porphyrin present in each fraction.

To evaluate the heme polymerization inhibitory activity (HPIA) of reducing agents, 100 μL of a 4 mM solution of hematin, dissolved in 0.1 M NaOH, was distributed in 96-well U-bottom microplates (0.4 μmol/well) (Costar 3799). Different doses of compounds were then added to quadruplicate test wells. In control wells, 50 μL of water was used. Hematin was precipitated by the addition of 0.8 mmol of acetic acid (50 μL) and the suspension was incubated for 24 h at 37 °C. At the end of incubation, the plates were processed as described above.

The IC_{50} was defined as the molar equivalent of test compound able to inhibit hematin polymerization by 50% *(18)*.

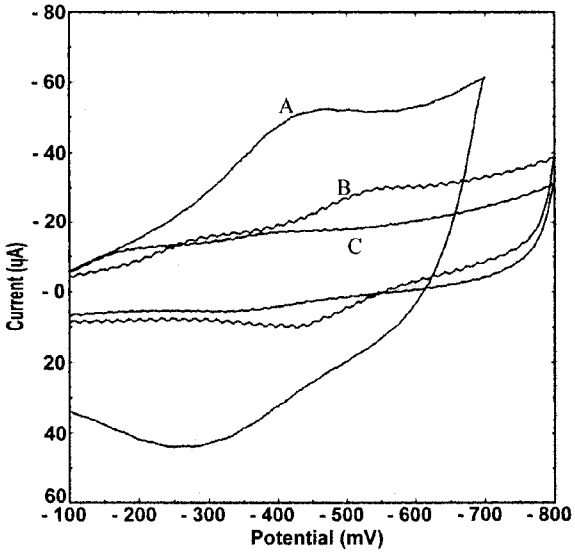

FIGURE 1: Electrochemical reduction of 5 mM Fe(III)PPIX in a preparative electrolytic cell (potential from −0.1 to −0.7 V/s; scan rate of 200 mV/s). Cyclic voltammograms at the beginning (A), during (B), and at the end of the electrolysis (C). Irregularities in the shape of voltammograms B and C are due to the fact that the reaction was under stirring conditions.

Parasite Cultures and Drug Sensitivity Assays. P. falciparum (chloroquine-resistant, W2 strain) was cultured using group A^+ human erythrocytes at 5% hematocrit in RPMI-1640 (HyClone, Cremlington, U.K.) supplemented with 10% heat-inactivated human plasma, 32 mM $NaHCO_3$, 25 mM Hepes, pH 7.4. Cultures were maintained at 37 °C in an atmosphere of 5% CO_2, 5% O_2, 90% N_2. Tests were carried out on nonsynchronized cultures, diluted to 1% parasitemia and 2% hematocrit, in a final volume of 2 mL in 24-well plates (Costar 3524) in duplicate. NAC and cysteine were added in different doses ranging from 0.1 to 20 mM, and chloroquine was used at a final concentration of 1 μg/mL = 1.9 μM. After 48/72 h of incubation, thin blood films were made from each well, stained with Giemsa, and the percentage parasitemia was calculated by microscopic examination.

RESULTS

Electrochemical Reduction of Fe(III)PPIX. Fe(II) PPIX was obtained in pure form and large quantities, in the absence of oxygen, by complete electrochemical reduction of a solution of Fe(III)PPIX. As shown in Figure 1, a 5 mM solution of hemin was reduced with a square wave at −0.6 V for 10 s and −0.55 V for 1 s to permit diffusion from the electrode. Progress of electrolysis was monitored by cyclic voltammetry directly in the preparative electrolytic cell from −0.1 to −0.7 V/s at scan rate of 200 mV/s. The reduction peak was observed at −0.5 V; the oxidation peak at −0.3 V indicates that the reaction is almost completely reversible (Figure 1, voltammogram a). The difference between the reduction and the oxidation peaks is due to partial electrode inactivation as the reaction proceeds. Irregularities in the shape of the trace of the voltammogram in the preparative cells stem from the fact that the reaction was carried out with stirring (Figure 1, voltammograms B and C).

In addition, the electrolysis was monitored over time by sampling the solution in the preparative cell and testing it in an analytical electrolytic cell. The results conformed with those previously published by Zhang et al. *(20).*

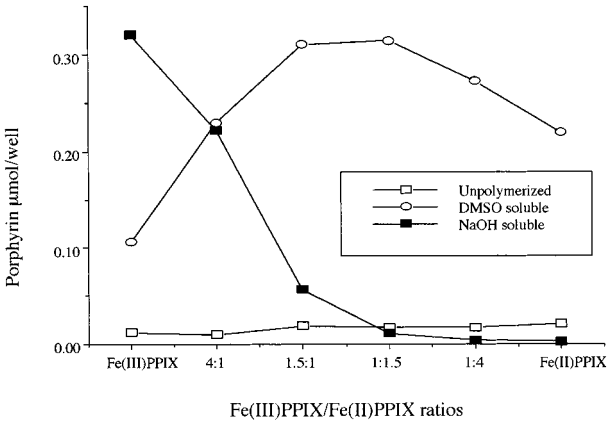

FIGURE 2: Inhibition of Fe(III)PPIX polymerization by different molar ratios of electrochemically reduced Fe(II)PPIX. The microassay of heme polymerization inhibitory activity (HPIA) was run under nitrogen for 24 h at room temperature. Note that the error bars fall within the boundaries of the symbols.

Inhibition of Hematin Polymerization by Fe(II)PPIX. Fe(II)PPIX was used immediately after completion of the electrochemical reduction in the standard acidic conditions of polymerization of Fe(III)PPIX *(10, 18).* To avoid reoxidation of Fe(II)PPIX, the entire polymerization reaction was carried out under strict nitrogen atmosphere. Using the HPIA microassay, a dose-dependent inhibition of hematin polymerization was observed with increasing amounts of Fe(II)PPIX added at the beginning of the reaction (Figure 2). The progressive decrease of the NaOH-soluble fraction (β-hematin) was paralleled by the increase in the DMSO-soluble fraction (unpolymerized hematin). No significant amounts of unprecipitated hematin were recovered. Under the same conditions, no polymerization was observed with Fe(II)PPIX alone. The calculated IC_{50} for Fe(II)PPIX was 0.5 molar equiv.

The analysis of the reaction products by FT-IR showed the disappearance of the peaks characteristic of β-hematin (at 1662 and 1209 cm^{-1}) at the IC_{50} (Figure 3).

Heme Polymerization Inhibitory Activity of Thiol-Containing Compounds. A dose-dependent inhibition of heme polymerization was obtained with different doses of L-cysteine, *N*-acetylcysteine (NAC), DL-homocysteine, cysteamine, or 2-mercaptoethanol (2-ME) (Figure 4). In contrast, no inhibition was found with the corresponding oxidized compound, such as cystine. Varying the concentrations of hematin in the HPIA assay did not modify significantly the molar ratios resulting in 50% inhibition of hematin polymerization: NAC was one of the most effective compounds with IC_{50}s of 0.16, 0.17, and 0.19 using 0.2, 0.4, and 0.8 μmol of hematin/well, respectively.

To verify that the observed inhibition of hematin polymerization with thiols was due to the reduction of Fe(III)-PPIX, the same solutions used for the HPIA were characterized by analytic cyclic voltammetry. The results are shown in Figure 5, panels A and B. In the absence of thiols, iron is completely oxidized [Fe(III)PPIX] (Panel A, voltammogram A1: no peak detected); when cysteine is added to the solution under nitrogen, an oxidation peak is detected (panel A, voltammogram A3) at approximately −200 mV, demonstrating the generation of reduced iron; the oxidation peak current decreases when oxygen is added as Fe(II)PPIX is oxidized

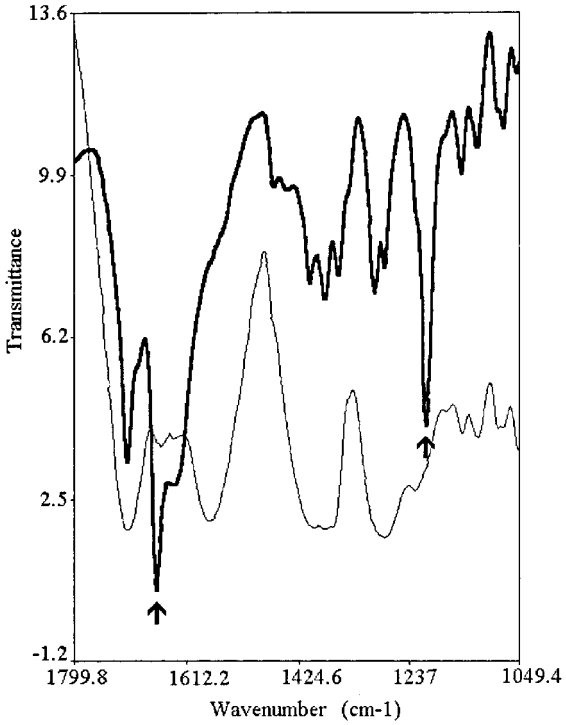

FIGURE 3: Infrared spectra of synthetic β-hematin obtained after 60 min incubation in 4.5 M acetate, pH 5, 60 °C (boldface line), and of hematin polymerized in the presence of 0.5 equiv of Fe(II)-PPIX under nitrogen (thin line). The characteristic peaks of β-hematin at 1662 and 1209 cm^{-1} are marked with arrows.

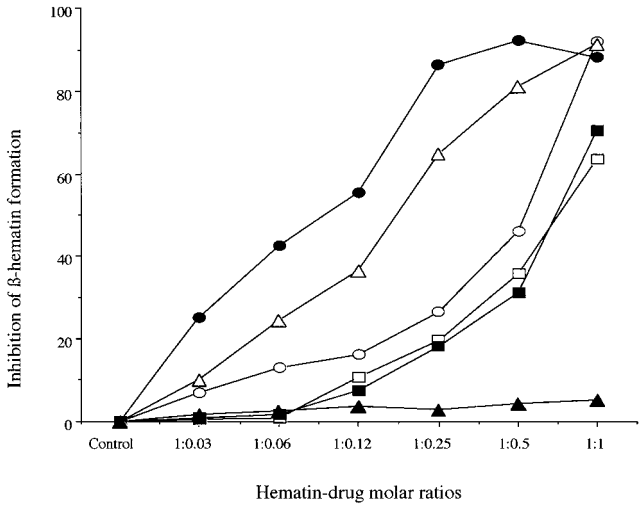

FIGURE 4: Inhibition of Fe(III)PPIX polymerization by different molar ratios of thiol-containing compounds. The microassay of heme polymerization inhibitory activity (HPIA) was done in acetic acid for 24 h at 37 °C. Details are described under Experimental Procedures and in ref *18*. The results are expressed as percent inhibition of β-hematin formation in the presence of reducing agents compared to hematin alone. L-Cysteine (○) (0.72 ± 0.22), *N*-acetylcysteine (●) (0.17 ± 0.06), DL-homocysteine (□) (0.86 ± 0.17), cysteamine (■) (0.74 ± 0.18), 2-mercaptoethanol (△) (0.20 ± 0.01), L-cystine (▲). Note that the error bars fall within the boundaries of the symbols. The IC_{50}s (in parenteses) were calculated as the molar equivalent of test compound able to inhibit hematin polymerization by 50% *(18)*.

(panel A, voltammogram A4). Similar results were obtained using NAC (Figure 5, panel B).

Different doses of NAC or cysteine were tested for their ability to inhibit the growth of *P. falciparum* (W2 strain) in vitro at 48 and 72 h. Both compounds were toxic for the

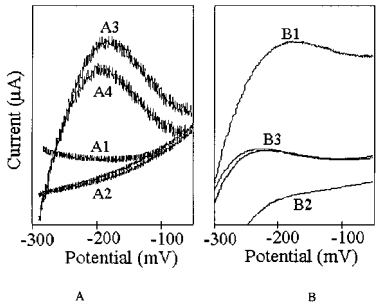

FIGURE 5: Cyclic voltammograms of Fe(III)PPIX in 4 M acetate buffer in the presence of cysteine (A) or *N*-acetylcysteine (B). (A) Line A1, hematin completely oxidized; line A2, cysteine alone; line A3, hematin + cysteine; the oxidation peak of hematin (−150 mV) is due to the presence of Fe(II)PPIX; line A4, as in A3 after inflow of air. (B) Line B1, hematin + NAC, the oxidation peak of hematin (−200 mV) is due to the presence of Fe(II)PPIX; line B2, NAC alone; line B3, as in B1 after inflow of air.

parasites in a dose- and time-dependent manner. At 48 h, 10 mM NAC or cysteine inhibited parasite growth by more than 50% (NAC = 59.7 ± 3.1%; cysteine = 58.8 ± 3.6%); at 72 h, the toxicity increased to 89.9 ± 4.1% and 81.7 ± 4.5% with 10 mM NAC or cysteine, respectively.

DISCUSSION

This is the first report of Fe(II)PPIX obtained at preparative scale by electrolysis in the quantities and purity needed for the HPIA; the resulting analytical voltammograms are consistent with prior reports *(20, 21)*. The analytical conditions of hematin electrolysis *(20, 21)* were adapted to the preparative cell: a larger platinum electrode allowed us to obtain larger quantities of reduced product under stirring conditions. The reaction was conducted under constant temperature and strict nitrogen atmosphere to avoid reoxidation of Fe(II)PPIX. Initially, electrochemical reduction was preferred over reducing agents to avoid chemical interference with the polymerization reaction.

Consistent with the properties of reduced iron—which does not allow the formation of the carboxylate bond needed to obtain β-hematin *(6, 10)*—Fe(II)PPIX did not polymerize in the HPIA assay in anaerobic conditions. Such findings also exclude the possibility that an internal dismutation involving ferrous iron and one vinyl group of the porphyrin could occur: this would result in the formation of Fe(III)PPIX and subsequent polymerization.

Fe(II)PPIX is as effective an inhibitor of Fe(III)PPIX polymerization as non-iron porphyrins (namely hematoporphyrin) and is more potent than chloroquine as measured by molar equivalents *(12)*. We propose that both (i) coplanar electronic $\pi-\pi$ interactions between Fe(II)PPIX and Fe(III)-PPIX—as in the case of quinoline antimalarials *(10)* and non-iron porphyrins *(12)*—and (ii) hydroxo bridge bonds between one molecule of Fe(II)PPIX and the axial hydroxylic group of two molecules of Fe(III)PPIX contribute to the stability of the complex (Figure 6). Hydroxo bridges form because Fe(II)PPIX can bind two molecules of H_2O in axial position at low pH *(22, 23)*. The role of hydroxo bridge bonds involving the hydroxyl group in the benzylic position is postulated for hematoporphyrin *(12)* and is demonstrated by Mossbauer spectroscopy for quinoline-containing compounds *(16, 24)*.

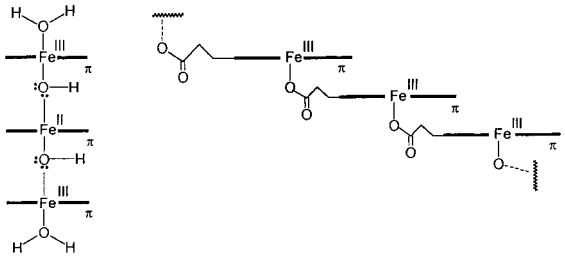

FIGURE 6: Schematic representation of the proposed structure of the Fe(II)/Fe(III)PPIX complex (right) and of β-hematin *(6)* (left). In the complex, one molecule of Fe(II)PPIX forms (a) coplanar $\pi-\pi$ interactions and (b) hydroxo bridge bonds with the axial hydroxylic groups of two molecules of Fe(III)PPIX. The boldface lines indicate the tetrapyrrole ring of the porphyrins.

The results obtained with Fe(II)PPIX point to a fundamental requirement for malaria parasites to survive: the need for complete oxidation of Fe(II)PPIX to Fe(III)PPIX to avoid inhibition of hematin polymerization.

If this is true, one would expect the process to be inhibited by reducing agents as well. This appeared to be the case with several thiol-containing compounds that proved able to inhibit hematin polymerization in a dose-dependent manner. Similar observations were recently reported *(9)*. That Fe(II)PPIX is generated was confirmed by cyclic voltammetry. This is consistent with previous results obtained using different techniques (Mossbauer, ESR, and absorption spectroscopies): in all cases, the final products of the reactions between thiols and ferric iron (including hematin iron) contain Fe(II) *(25, 26)*. Rapid reduction of iron was reported at low pH with L-cysteine, NAC, or glutathione *(27)*. It is worth noticing that glutathione has recently been implicated in the degradation of unpolymerized heme in parasitized red blood cells: the reaction requires the presence of oxygen and Fe(III)PPIX, and it is likely to occur via the generation of Fe(II)PPIX *(5)*.

These findings suggest that any reducing compound (currently being investigated in our laboratory) with a redox potential compatible with the Fe(II)/Fe(III) redox couple (ca. -0.3 mV) could reduce Fe(III)PPIX, thus preventing its polymerization. In our experimental conditions, the reaction kinetics favor Fe(III) reduction over Fe(III) polymerization.

Inhibition of parasite growth in vitro was observed with some of the thiols tested, albeit this did not completely correlate with the HPIA activities. This was not entirely unexpected since: (i) the availability and accumulation of these drugs in the parasite food vacuole are at present unknown and these molecules are potentially prone to inactivation due to interaction with parasite metabolic pathways prior to reaching their target in the food vacuole; (ii) the range of pHs of the parasite's food vacuole (approximatively pH 5.5) *(28)* dictates the amount of Fe(III) reduced at any one time, since the redox potential of the thiols is pH dependent *(27, 29, 30)*; and (iii) at least two competing reactions are likely to occur in the food vacuole: Fe(III)-PPIX reduction by thiols versus heme iron-catalyzed oxidation of thiols by oxygen, which lowers the availability of reduced compounds.

In this context, thiol compounds present in the food vacuole appear to be a double-edged sword for parasite survival: (a) as anti-oxidants, they are needed to scavenge the oxygen radicals generated during the oxidation of hemoglobin-derived iron; (b) as reducing agents, by reducing Fe(III) to Fe(II)PPIX, they inhibit the detoxification of hematin and increase the generation of hydroxyl radicals and hydrogen peroxide with consequent lipid peroxidation *(4, 31, 32)*.

Current research in our laboratory focuses on reducing compounds that will be more appropriate and effective antimalarials: compounds that, unlike thiols, are not oxygen scavengers and are not susceptible to iron-catalyzed oxidation of the functional groups.

In conclusion, effective inhibition of heme polymerization can be achieved by simply modifying the oxidative status of porphyrin iron. Small amounts of Fe(II)PPIX are necessary for the activation of the peroxide bridge of artemisinin-type molecules *(33, 34)*. Fe(II) is also involved in the lipid peroxidation process via interaction with vinyl groups to form epoxides *(35)*. Therefore, we should expect that compounds able to reduce Fe (III) to Fe(II) could synergize with arthemisinin-related compounds by generating enough Fe(II)-PPIX necessary for the activation of artemisinin and for the inhibition of heme polymerization.

Further applications of such compounds alone as well as in a combined therapy together with artemisinin derivatives are envisaged.

ACKNOWLEDGMENT

We are thankful to Prof. M. Ghione, Prof. Omodeo Salé, and Dr. T.J. Egan for invaluable advice and helpful comments. We thank Dr. Ravagnani from the Haematology Unit, National Cancer Institute, Milan, Italy, for providing the red blood cells used for growing *P. falciparum* parasites.

REFERENCES

1. Goldberg, D. E., Slater, A. F. G., Cerami, A., and Henderson, G. B. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 2931−2935.
2. Olliaro, P., and Goldberg, D. (1995) *Parasitol. Today 11*, 294−297.
3. Francis, S. E., Sullivan, D. J., and Goldberg, D. E. (1997) *Annu. Rev. Microbiol. 51*, 97−123.
4. Golenser, J., Marva, E., and Chevion, M. (1991) *Parasitol. Today 7*, 142−146.
5. Ginsburg, H., Famin, O., Zhang, J., and Krugliak, M. (1998) *Biochem. Pharmacol. 56*, 1305−1313.
6. Slater, A. F. G., Swiggard, W. J., Orton, B. R., Flitter, W. D., Goldberg, D. E., Cerami, A., and Henderson, G. B. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 325−329.
7. Dorn, A., Stoffel, R., Matile, H., Bubendorf, A., and Ridley, R. (1995) *Nature 374*, 269−271.
8. Sullivan, D., Gluzman, I., and Goldberg, D. (1996) *Science 271*, 219−222.
9. Dorn, A., Vippagunta, S., Matile, H., Bubendorf, A., Vennerstrom, J., and Ridley, R. (1998) *Biochem. Pharmacol. 55*, 737−747.
10. Egan, T., Ross, D., and Adams, P. (1994) *FEBS Lett. 352*, 54−57.
11. Bohle, S., Dinnebier, R. E., Madsen, S. K., and Stephens, P. W. (1997) *J. Biol. Chem. 272*, 713−716.
12. Basilico, N., Monti, D., Olliaro, P., and Taramelli D (1997) *FEBS Lett. 409*, 297−299.
13. Chou, A. C., Chevli, R., and Fitch, C. D. (1980) *Biochemistry 19*, 1543−1549.
14. Sullivan, D., Gluzman, I., Russell, D., and Goldberg, D. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 11865−11870.
15. Moreau, S., Perly, B., and Biguet, J. (1982) *Biochimie 64*, 1015−1025.
16. Adams, P., Egan, T., Ross, D., Silver, J., and Marsh, P. (1996) *Biochem. J. 318*, 25−27.
17. Marques, H., Voster, K., and Egan, T. (1996) *J. Inorg. Biochem. 64*, 7−23.

18. Basilico, N., Pagani, E., Monti, D., Olliaro, P., and Taramelli, D. (1998) *J. Antimicrob. Chemother. 42*, 55−60.
19. Bednarski, T., and Jorda, J. (1967) *J. Am. Chem. Soc*. 89, 1552−1558.
20. Zhang, F., Gosser, D., and Meshnick, S. (1992) *Biochem. Pharmacol. 43*, 1805−1809.
21. Chen, Y., He, C. X., Zhu, S. M., Chen, H. Y., and Li, Y. (1997) *J. Electrochem. Soc. 144*, 1891−1894.
22. Dawson, R. M. C., Eliott, D. C., Eliott, W. H. and Jones, K. M. (1969) *Data for Biochemical Research*, Clarendon Press, Oxford.
23. Das, D.K., and Medhi, O.K. (1998) *J. Inorg. Biochem. 70*, 83−90.
24. Bhattacharjee, A., and Karle, J. (1996) *J. Med. Chem. 39*, 4622−4629.
25. Wagnerovà, D. M., Stopka, P., and Lassmann, G. (1984) *Collect. Czech. Chem. Commun. 49*, 2579−2585.
26. Bayer, E., Hill, H. A. O., Roder, A., and Williams, R. J. P. (1969) *Chem. Commun.* 109.
27. Hamed, M. Y., Silver, J., and Wilson, M. T. (1983) *Inorg. Chim. Acta 78*, 1−11.
28. Goldberg, D., and Slater, A. (1992) *Parasitol. Today 8*, 280−283.
29. Jamenson, R. F., Linert, W., Tschinkowitz, A., and Gutmann, V. (1988) *J. Chem. Soc., Dalton Trans.*, 943−946.
30. Sakurai, H., Hatayama, E., and Nishida, M. (1983) *Inorg. Chim. Acta 80*, 7−12.
31. Omodeo-Sale', F., Basilico, N., Folini, M., Olliaro, P., and Taramelli, D. (1998) *FEBS Lett. 433*, 215−218.
32. Schirmer, R. H., Muller, J. G., and Krauth-Siegel, R. L. (1995) *Angew. Chem. Int. Ed. Engl. 34*, 141−154.
33. Posner, G., Cumming, J., Ploypradith, P., and Chang, HO, Oh (1995) *J. Am. Chem. Soc. 117*, 5885−5886.
34. O'Neill, P., Bishop, L., Storr, R., Hawley, S., Maggs, J., Ward, S., and Park, B. (1996) *J. Med. Chem. 39*, 4511−4514.
35. Tang, L. X., Yang, J. L., and Shen, X. (1997) *J. Inorg. Biochem.*, 265−272.

BI990085K