Redox Reaction of Artemisinin with Ferrous and Ferric Ions in Aqueous Buffer

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Artemisinin, a sesquiterpene with endoperoxide bond, possesses potent antimalarial activity against the ring and late stage of chloroqine-resistant *Plasmodium falciparum* malaria both *in vitro* and *in vivo*. The mode of antimalarial activity of artemisinin is iron-dependent. The aim of this study was to investigate the reactions of artemisinin with ferrous and ferric ions in aqueous buffer. Artemisinin generated a cycle of iron oxidation and reduction. It oxidized ferrous and reduced ferric ions with similar rate of reaction ($k=10\pm0.5\ \mathrm{M^{-1}\cdot s^{-1}}$ for ferrous and $k=8.5\pm2.0\ \mathrm{M^{-1}\cdot s^{-1}}$ for ferric ion). The major active product was dihydroartemisinin which exhibited antimalarial activity at least 3 times more potent than artemisinin. Dihydroartemisinin preferably binds to ferric ion, forming ferric–dihydroartemisinin complex. The re-oxidation of the complex gives artemisinin and ferric ion. This suggests that in aqueous buffer, the reaction of artemisinin with iron may give rise to the active reaction products, one of them being dihydroartemisinin, which is responsible for antimalarial activity.

Key words artemisinin; iron; dihydroartemisinin; redox; antimalarial activity

Artemisinin (qinghaosu), a sesquiterpene lactone with endoperoxide bond, possesses potent antimalarial activity against chloroqine resistant *Plasmodium falciparum*, widely used for therapy of malaria in China and Southeast Asia. It was demonstrated that artemisinin can kill parasites in the ring and late stage, both *in vitro* and *in vivo*.[1)] It is believed that the mode of action of artemisinin is its iron-dependent mechanism, involving cleavage of the peroxide bridge by heme iron, yielding carbon centred free radicals[2—4)] which in turn alkylates some parasite-specific proteins.[5)] Although the chemical reaction of artemisinin with free iron or heme has been extensively studied, and the end-products have been characterized in many laboratories,[6—13)] the nature of the biological active intermediates is still unclear.

Many biomolecules are well suited for metal chelation and are considered to be prooxidant or antioxidant or both (*e.g.* ascorbate).[14)] The prooxidant activity of these compounds is related to the production of reactive oxygen species such as $O_2^{\cdot-}$ or H_2O_2. This event occurs in the presence of transition metals.[14,15)] Coordination of transition metals to biomolecules almost always involve *d* orbitals of the metal. In addition, dioxygen can also ligate to transition metals primarily through the *d* orbitals of the metals. Therefore, transition metals may simultaneously bind to biomolecules and dioxygen and may often act as a bridge between the molecule and dioxygen, for example, the formation of active O_2–Fe^{2+}–bleomycin as an intermediate in bleomycin-induced DNA cleavage.[16)]

In this study, we have shown that in an aqueous buffer artemisinin generates a cycle of iron oxidation–reduction reaction and consumption of dioxygen, yielding an active product, dihydroartemisinin. Dihydroartemisinin binds favorably to ferric ion, yielding an intermediate, ferric–dihydroartemisinin complex. The complex is oxidized in the presence of oxygen, yielding artemisinin.

Experimental

Chemicals Artemisinin was purchased from Sigma and its purity was checked by ^{1}H-NMR. Dihydroartemisinin was kindly provided by Professor Yodhathai Thebtaranonth, Department of Chemistry, Mahidol University, Thailand. $FeSO_4\cdot 7H_2O$ was from Carlo Erba Reagenti. $FeCl_3$ anhydrous was from Fluka Chemika. Chelex 100 resin was from Bio-Rad Laboratories. 1,10-Phenanthroline hydrate (ϕ) was from Ajak Chemicals. TRIZOL® reagent was from Life Technologies. All reagents were of the highest quality available.

Decontamination of Adventitious Metals and Buffer Used The presence of contaminating metals in reagents can lead to unpredictable change in the redox chemistry of iron under study due to exchange of contaminating metals with iron, which may affect the rates of oxidation or reduction of iron. Therefore, in order to remove trace amounts of adventitious catalytic metals in water, chelating resin (Chelex 100 resin) was used as described previously.[14,15)] Chelex 100 resin was added into double-distillated water (5 mg/100 ml) and stirred gently for 1 h. Then, the water was filtered from the resin using filter paper for quantitative analyses (MN 615, Macherey-Nagel).

The buffer solutions were prepared in glassware. The glassware was washed with 10% nitric acid and rinsed three times with metal-free water. The buffer solution contained 20 mM Hepes buffer plus 132 mM NaCl, 3.5 mM KCl, 0.5 mM $MgCl_2$, and 5 mM glucose, pH 7.25.

All other reagents were prepared in disposable plastic wares. The stock solution of 0.01 M ferrous or ferric ions (in 0.4 M H_2SO_4) was freshly prepared before being used. The stock solutions of 0.01 M artemisinin were prepared in dimethyl sulfoxide (DMSO) and stored at −20 °C. To obtain the desired concentration of artemisinin, the stock solution was diluted with a buffer.

The absorption spectra were recorded on a Hewlett Packard HP 8435 and a Shimadzu, UV 2501 PC spectrophotometer. Experiments were conducted in a 1-cm quartz cuvette containing 2 ml of solution under continuous stirring. The temperature was controlled at 25 °C using a peltier temperature control, cell holder model 89090A. The ^{1}H-NMR spectra were recorded on a Brucker model WM 250 spectrometer. The mass spectra were recorded on an API 100 spectrometer.

Determination of Ferrous and Ferric Concentration Ferrous concentration was determined by colorimetric method, using 1,10-phenanthroline as chelator. The reaction was performed in 50 ml air-saturated or N_2-saturated buffer solution. At desired time intervals, 2 ml of the reaction mixture were removed and added to 0.1 ml of 25 mM 1,10-phenanthroline in 0.25 M sodium acetate buffer pH 5.3 containing 50 mM sodium arsenite. The ferrous–phenanthroline complex was monitored by the absorbance at 515 nm with the molar extinction coefficient (ε) equal to 11000 $\mathrm{M^{-1}\cdot cm^{-1}}$.[17)]

$$Fe^{2+}+3\phi \rightarrow [Fe\cdot 3\phi]^{2+}$$

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Ferric ion concentration was determined by spectrophotometric method. The lyophilized products were dissolved in 0.4 M H_2SO_4. The organic compounds were eliminated by addition of 0.01 M HCl. The concentration of ferric ion was determined using molar extinction coefficient, ε (λ=304 nm) equal to 2204 $M^{-1} \cdot cm^{-1}$.[18] Under the conditions of performing reaction (buffer, pH 7.3) before adding artemisinin, ferric ion was stable and its oxidation did not occur.

Characterization of Reaction Products Two milliliters of the mixture solutions of artemisinin with ferrous or ferric ions were removed at the desired time. Then, the reaction was stopped by extraction with chloroform, and the ^{1}H-NMR, mass spectrometry and UV absorption spectra of products were recorded. Furthermore, the reaction products were confirmed using silica gel TLC (thin layer chromatography), and plated in a saturated chamber containing chloroform/acetone (98 : 2) as mobile phase. The spots of artemisinin and its reaction products were detected by coloring with iodine.

Culture of *Plasmodium falciparum* The *Plasmodium falciparum* strain TM267R was cultured in RPMI1640 completed with 10% human heat-inactivated serum, containing 25 mM Hepes, 22 mg/ml $NaHCO_3$, 1.8 mg/ml D-glucose and 40 μg/ml gentamicin, and human blood group O erythrocytes at 37 °C in a humidified atmosphere with 5% CO_2.[19] The medium was changed daily. The parasite count and morphology were examined every day by thin blood smear. Ring stage-infected erythrocytes were obtained by synchronization using 5% D-sorbitol.[20]

Antimalarial Activity of Artemisinin and Its Reaction Products The cultures were initiated at 2.5×10^6/ml of ring stage-infected erythrocytes in 4 ml of the medium. They were exposed to various concentrations of artemisinin and its reaction products (0.001% final concentration of organic solvent). After 20 h of drug exposure, the inhibition of DNA synthesis was determined as the parameter of antimalarial activity. DNA was isolated using a mono-phasic solution of phenol and guanidine isothiocyanate (TRIZOL® reagent).[21] One ml of TRIZOL® reagent was added in cell suspension with the final cell count equal to 10^7 cells/ml, which was then incubated for 5 min at room temperature. Next, 0.2 ml of chloroform was added, the sample mixtures were shaken vigorously for 15 s, and incubated at room temperature for 2—3 min. The mixtures were centrifuged at less than 12000×***g*** for 15 min at 2—8 °C, and the colorless upper phase was discarded. Then, 0.3 ml of absolute ethanol was added to the interphase and red lower phenol–chloroform phase, and they were mixed. Next, the samples were incubated at room temperature for 2 min, and centrifuged at 2000×***g*** for 5 min at 2—8 °C to sediment the DNA. Afterwards, the phenol–ethanol supernate was removed, and the DNA pellet was washed twice in 1 ml of the solution containing 0.1 M sodium citrate in 10% ethanol. At each wash, the DNA pellet was stored in the washing solution for 30 min at room temperature with periodic mixing, and centrifuged at 2000×***g*** for 5 min at 2—8 °C. After washing, the DNA pellet was suspended in 75% ethanol (1.5—2 ml), stored for 10—20 min at room temperature, and centrifuged at 2000×***g*** for 5 min at 2—8 °C. DNA suspended in 75% ethanol can be stored at 2—8 °C for months.

To redissolve the DNA, the DNA pellet was dried by N_2 and dissolved in 150 μl of 8 mM NaOH. The DNA content was determined by measurement of fluorescence intensity of the DNA–ethidium bromide complex at excitation 478 nm and emission 600 nm (recorded by Perkin Elmer Luminescence LS50B). Seventy microliters of DNA sample dissolved in 8 mM NaOH was added in a 2-ml cuvette containing 40.6 μM ethidium bromide in 0.05 M K_2HPO_4/NaH_2PO_4 buffer, pH 7.5 with continuous stirring at room temperature.

The IC_{50} was determined by plotting the percentage of DNA synthesis *versus* the logarithm of the concentrations of the compounds. IC_{50} is the drug concentration that inhibits DNA synthesis by 50%. The relative antimalarial activity (IC_{50}) of products compared to that of artemisinin was calculated.

Results

Characterization of Reaction Products The reaction of artemisinin and ferrous ions was performed in buffer solution, pH 7.25 at 25 °C. The reaction products of the equimolar solution, [artemisinin]=[ferrous]=100 μM, were extracted by chloroform at the desired times and identified by TLC plate. Figure 1 shows the typical characteristic migration of artemisinin, dihydroartemisinin, and the reaction products of artemisinin with ferrous or ferric ions. The characteristic migration (*Rf*) of artemisinin and dihydroartemisinin were 0.68 and 0.14, respectively. It was confirmed that the artemisinin was stable in the buffer solution (pH 7.3) for at least 2 h. In contrast, artemisinin disappeared rapidly after addition of ferrous or ferric ions. Two new characteristic bands of reaction products appeared at *Rf* 0.07 and 0.14. The compound with *Rf* 0.14 was characterized by mass spectrometry, corresponding to dihydroartemisinin (*m*/*z*=284). Four hours later the characteristic band of artemisinin (*Rf* 0.68) reappeared.

Fig. 1. The Characteristic Migration of Artemisinin (1), Dihydroartemisinin (2), the Reaction Products at 20 s (3), and 4 h (4) of the Reaction of Artemisinin with Ferrous Ion; and the Reaction Products at 20 s (5) and 4 h (6) of the Reaction of Artemisinin with Ferric Ion on TLC (Silica Gel)

A solution containing 0.1 mM of artemisinin or dihydroartemisinin was prepared in buffered solution pH 7.25 at 25 °C and extracted by chloroform. The reaction of equimolar solution ([artemisinin]=[ferrous] or [artemisinin]=[ferric]=0.1 mM) was performed in buffer solution pH 7.25 at 25 °C. The reaction products were extracted by chloroform. The chloroform phase was discarded and spotted on TLC plate and developed in a saturated chamber containing chloroform/acetone (98 : 2) as mobile phase. The spots of molecules were detected by coloring with iodine.

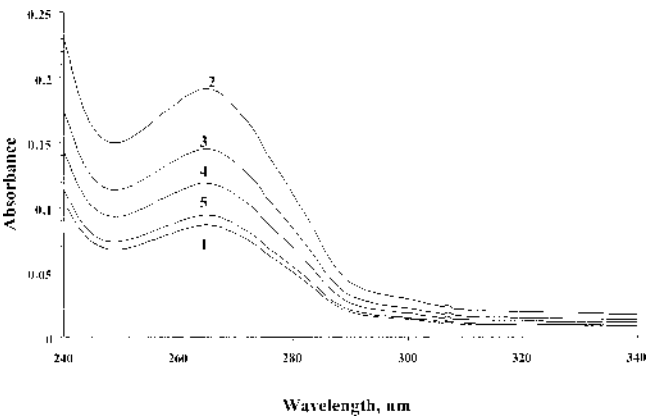

Fig. 2. Absorption Spectra of the Reaction Products Extracted by Chloroform at 20 s (1), 10 min (2), 12 min (3), 14 min (4), and 15 min (5)

The reaction solution contained equimolar concentration of artemisinin and ferrous ion (0.1 mM) in buffer solution pH 7.25 at 25 °C.

In order to characterize the compound with the *Rf* 0.07, the reaction products from the equimolar solution, [artemisinin]=[ferrous]=100 μM, were extracted by chloroform at desired times after performing the reaction. It should be noted that neither artemisinin nor dihydroartemisinin in chloroform absorbed UV/vis. light. The extracted solutions gave the characteristic absorption band at 266 nm (Fig. 2), which behaved as a sine curve. It showed a maximal absorption at 10 min and minimal absorption at 15 min, indepen-

dent of the initial concentration of both reactants. Under this condition, the reaction formed with 1 : 1 stoichiometry, giving an intermediate complex which has molar extinction coefficient at 266 nm equal to $2600 \pm 40\ \text{M}^{-1} \cdot \text{cm}^{-1}$.

To understand the nature of light absorption, a series of experiments were performed; the initial concentration of artemisinin was fixed at 50 μM while the ferrous concentrations were varied from 1 to 100 μM. At 10 min after performing the reaction, the reaction products were extracted by chloroform. The chloroform phase was concentrated and dried under N_2 atmosphere. The dried products were redissolved in 0.4 M H_2SO_4 and 0.01 M HCl. The solutions possessed an absorption spectrum with the maximal absorbance at 304 nm, the characteristic absorption of ferric ion in 0.4 M H_2SO_4. Artemisinin did not show any absorption spectrum indicating the existence of a ferric–artemisinin complex.

Next, the variation of the absorbance at 304 nm as a function of the ratio of artemisinin to ferrous ion was demonstrated (Fig. 3). The absorbance at 304 nm (ferric ion) increased with the ratio of [artemisinin] : [Fe^{2+}] and reached a plateau when the ratio was equal to 1 : 1. This suggested that the complex formation would reach equilibrium with stoichiometry equal to 1 : 1. To confirm the complex formation, the reaction products were analyzed by ^{1}H-NMR. These products did not dissolve in water; they were twice redissolved in chloroform and repurified by adding buffer solution. The ^{1}H-NMR spectra of the reaction products from the reaction of artemisinin with ferrous and ferric ions (equimolar concentration) were very similar to that from commercial artemisinin (the singlet peak at δ 5.83 and multiplet peak at σ 3.2—3.4). There was no effect of iron on ^{1}H-NMR spectra, possibly due to removal of the iron during chloroform extraction and recrystallization. During the experimental procedures, the complex should be dissociated leading to artemisinin; however, this indicated that the molecule was still intact. In addition, analysis by mass spectrometry of artemisinin (m/z=283) and the reaction product showed that the reaction of artemisinin with ferrous and ferric ions gave a major product whose m/z was equal to 284.

Reaction in N_2-Saturated System To inhibit oxidation of the molecule, the reaction of artemisinin with ferrous or ferric ions was performed in nitrogen-saturated buffer. At 20 s, 1, 2, and 4 h, the reaction was stopped by addition of nitrogen-saturated chloroform. The aqueous phase was discarded and the chloroform phase was spotted on TLC plates. There was no band at Rf=0.68 at least during the time period of the experiments, suggesting that there was no reoxidation of products to artemisinin in the absence of dioxygen.

The Effect of Ferrous Concentration on the Reaction Equimolar Solution: Throughout the series of the experiments, the initial concentration of ferrous ion and artemisinin were varied from 0.5 to 200 μM.

In an aqueous solution, ferrous ion was susceptible to autoxidation to form ferric ion. The autoxidation in the buffer solution was checked by monitoring the remaining ferrous ion. In the buffer solution (pH 7.3) at 25 °C, the kinetics of change in ferrous concentration was demonstrated (Fig. 4). During the first 5 min, the concentration of ferrous ion was slightly changed in the absence of artemisinin, and thereafter it progressively decreased to remain about 20% after 35 min. The rate of autoxidation decreased at the lower temperature, or at pH below 7.3.

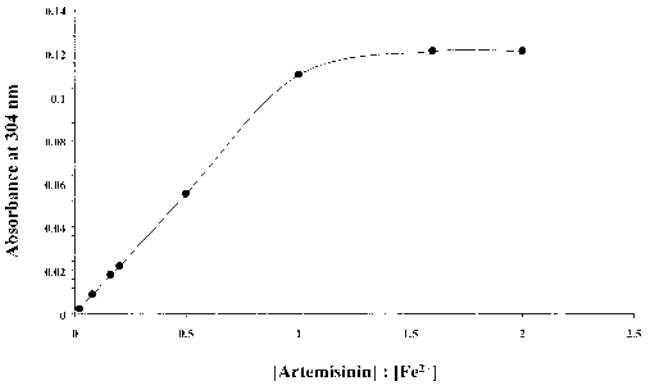

Fig. 3. Variation of Absorbance at 304 nm as the Function of the Ratio of [Artemisinin] : [Fe^{2+}]

The reaction solution contained equimolar concentration of artemisinin and ferrous ion (0.1 mM) in buffer solution pH 7.25 at 25 °C. 10 min after mixing, the reaction products were extracted by chloroform, evaporated and dried by blowing with nitrogen air. The dried products were redissolved in 0.4 M H_2SO_4 and 0.01 N HCl, and then the characteristic absorbance of ferric ion at 304 nm were determined.

The remaining ferrous concentration in the presence of artemisinin was also monitored over time (Fig. 4), and was found to decrease rapidly throughout the reaction. The change in concentration in the presence of artemisinin appeared to be a two-step process. In the first step, the ferrous concentration progressively decreased for 5 min. This change followed the first-order kinetics law, with a rate constant of $10 \pm 0.5\ \text{M}^{-1} \cdot \text{s}^{-1}$ (inset of Fig. 4). In the second step, the ferrous ion had reduced to about 10% of the initial amount after 10 min of reaction.

Our data showed that the reduction of artemisinin could be related to the oxidation of ferrous ion. The reduced form of artemisinin (dihydroartemisinin) bound favorably to ferric ion.

Reduction of Ferric Ion in the Presence of Artemisinin To verify the oxidant properties of artemisinin, a series of experiments was performed in equimolar concentrations of ferric ion and artemisinin. Throughout the series, the initial concentrations of ferric ion and artemisinin were varied from 0.5 to 200 μM.

The stock of ferric ions did not contain ferrous ions, as tested by colorimetric method using 1,10-phenanthroline. In the absence of artemisinin, no ferrous ion was detected, while in its presence, ferrous ion was formed rapidly (Fig. 5). Immediately after the mixing of ferric ion and artemisinin (equimolar concentration, 100 μM), 16 μM of ferrous ion was detected. The ferrous concentration decrease rapidly in a similar manner to that of the series of reactions of ferrous–artemisinin, reaching a pseudo-plateau with a remaining ferrous concentration of 8 μM. The final solution was colorless. At a high concentration of ferric and artemisinin (200 μM), the solution appeared to be yellow; some yellow precipitates, but no crystal.

The product of ferric–artemisinin reaction was extracted by chloroform and the absorption spectra were recorded. The product(s) displayed a UV absorption spectrum with maximum absorbance at 266 nm, identical to that of the ferrous–artemisinin reaction. Its maximal and minimal absorption were observed at 1 and 2 h, respectively, independent of the initial concentration. From TLC (Fig. 1), it appeared that the

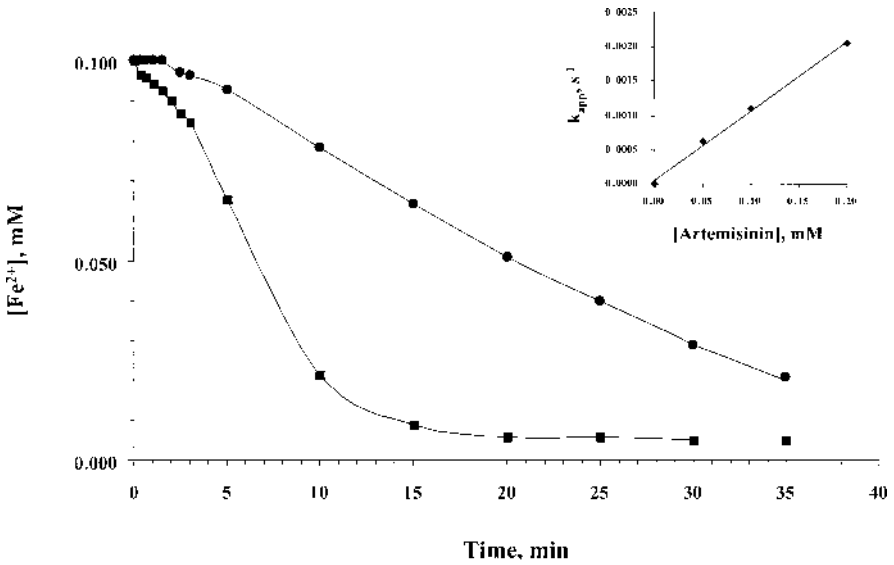

Fig. 4. Evolution of the Remaining Ferrous Concentration in the Absence (●) or in the Presence (■) of Artemisinin ([Artemisinin]=[Ferrous]=0.1 mM) in Buffer Solution pH 7.25 at 25 °C

Two milliliters of reaction mixture were removed and added to 0.1 ml of 25 mM 1,10-phenanthroline in 0.25 M sodium acetate buffer pH 5.3 containg 50 mM sodium arsenite. The ferrous–phenanthroline was monitored by absorbance at 515 nm with the molar extinction coefficient (ε) equal to 11000 $M^{-1} \cdot cm^{-1}$.

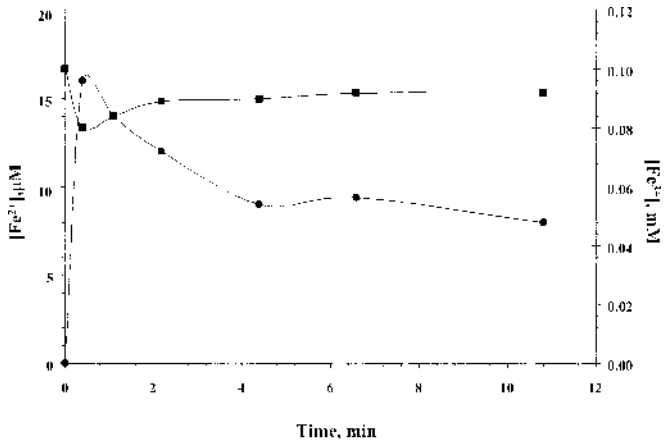

Fig. 5. Evolution of the Concentration of Remaining Ferric (■) and Occurring Ferrous (●) Ions in Buffer Solution Containing Equimolar Concentration of Artemisinin and Ferric ion (0.1 mM) pH 7.25 at 25 °C

intermediate species and the product were identical to those obtained from the reaction of artemisinin and ferrous ion.

In the presence of artemisinin, ferric concentration decreased with time reaching a pseudo-plateau after about 10 min (Fig. 5), independent of the initial concentration. This suggested that in the presence of artemisinin, ferric was rapidly reduced to ferrous, and then ferrous was oxidized to ferric. The apparent rate constant (k_{app}) of reaction could be analyzed according to a first-order kinetics law. The linear relationship of the apparent rate constant and the concentrations of artemisinin gave the second-order with a rate constant equal to $8.55 \pm 2.00\ M^{-1} \cdot s^{-1}$.

Antimalarial Activity of the Reaction Products The antimalarial activity of the intermediate species and the reaction product was tested comparing to artemisinin. Artemisinin was freshly prepared in DMSO and further diluted in RPMI1640 medium to obtain the desired concentrations. To obtain the reaction products, the reactions between

Table 1. Antimalarial Activity of Artemisinin and the Reaction Products of Artemisinin with Ferrous Ion[a)]

	Relative antimalarial activity
Artemisinin	1
Reaction product at 20 s	3.79±1.15
Reaction product at 4 h	4.83±1.05

a) The reaction of artemisinin and ferrous ion (equimolar concentration, 0.1 mM) was performed in nitrogen-saturated buffer pH 7.25 at 25 °C. The reaction products were extracted by nitrogen-saturated chloroform, concentrated and dried under nitrogen atmosphere. The molecules were incubated in parasite cultures (ring-stage) for 24 h. The antimalarial activity of the products was compared to that of artemisinin. Values are mean±S.D. (n=3).

artemisinin and ferrous ion at equimolar concentration (100 μM) were performed in nitrogen-saturated buffer and extracted by nitrogen-saturated chloroform. The reaction products were extracted at 20 s and at 4 h after mixing and were tested by TLC.

For light microscopy, thin blood films were made to observe the morphology of the infected erythrocytes. After exposure to artemisinin and the reaction products for 20 h, some parasites at ring stage had exhibited pyknotic nuclei and dense cytoplasm when the concentration was 0.1 nM or more. When the concentration was 10 nM, the parasites were completely dead, as the nuclei had formed clumps. The IC_{50} of artemisinin was 9.28±2.29 nM. The relative antimalarial activity of the products at 20 s and 4 h was at least 3 times more than artemisinin (Table 1).

Discussion

The interaction of artemisinin with ferrous and ferric ions was studied here in aqueous buffer in an attempt to determine the nature of the reaction, products and kinetics and thereby identify the mechanism of its action. The active metabolite of artemisinin in human plasma was characterized by HPLC as dihydroartemisinin.[22—24)] Our findings agreed with the data that dihydroartemisinin was at least three times

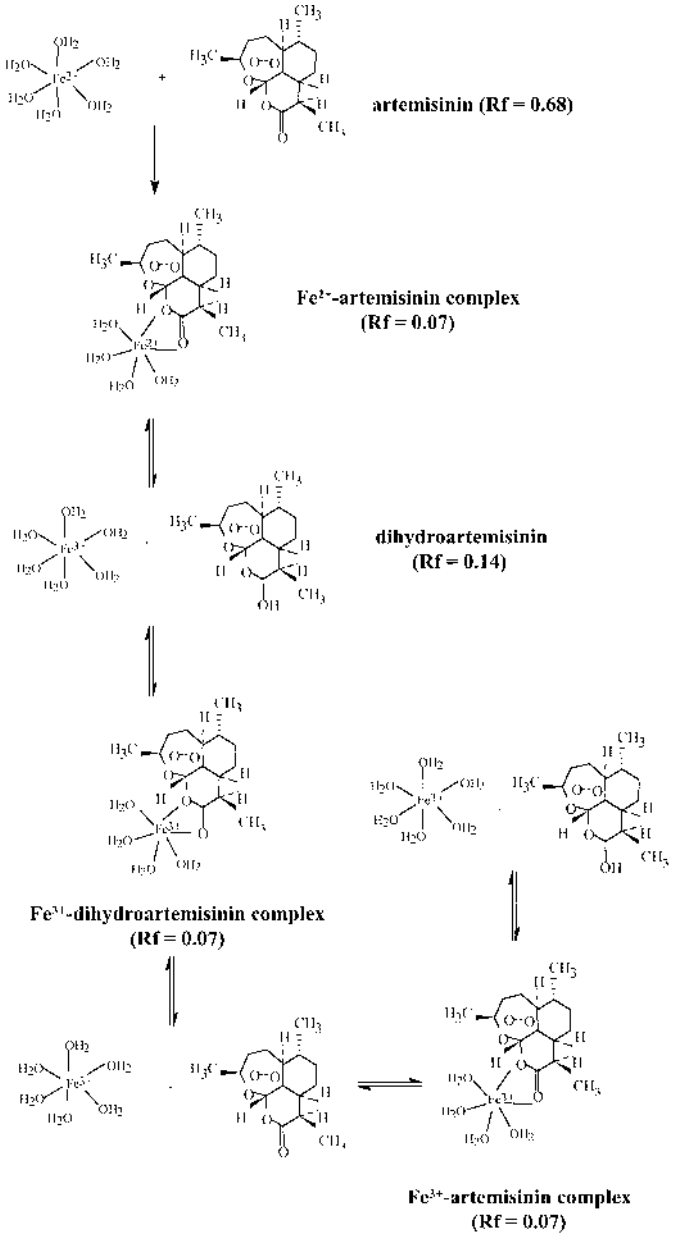

Chart 1. Proposed Reaction Mechanism of Artemisinin with Iron in Aqueous Solution

more potent than artemisinin, against *Plasmodium falciparum*.[25—27] Herein, the mechanism of artemisinin is proposed (Chart 1). In aqueous physiological buffer, artemisinin oxidized fer-rous and reduced ferric ion at a very similar rate. Dihydroartemisinin, the reduced and more active form of ar-temisinin preferably binds to ferric ion. Ferric ion serves as an electron bridge between dioxygen and dihydroartemisinin, yielding oxidation of the molecule, which is followed by dissociation of the complex, giving artemisinin.

In the ferrous–artemisinin reaction, a two-step mechanism is proposed herein. In the first step, ferrous ion would bind rapidly to artemisinin with 1 : 1 stoichiometry. The second-order limiting step of reaction with the mean rate constant equal to $10 \pm 0.5\ \mathrm{M}^{-1} \cdot \mathrm{s}^{-1}$ could be the one electron transfer, yielding the lactone reduction as proposed previously,[12] which could be followed by an intramolecular rearrangement, yielding dihydroartemisinin and ferric ion. The second step could be the formation of ferric–dihydroartemisinin complex. Oxidation of this complex would occur in the presence of dioxygen, yielding artemisinin. Next, ferric ion would bind to artemisinin to form a ferric–artemisinin complex, which would be reduced to form dihydroartemisinin and ferric ion. This is similar to the metal-catalyzed oxidation of biomolecules. The oxygen, nitrogen and sulphur atoms of biomolecules bind or form a bridge to transition metals. The true autoxidation of biomolecules such as ascorbate, dopamine, flavins, or diuric acid, is negligible in the absence of transition metal. These molecules undergo significant oxidation in the presence of (the transition metals) copper or iron.[15] Although dioxygen cannot react with biomolecules at a significant rate, many transition metals relieve the spin restriction of dioxygen and enhance the oxidation rate of biomolecules.[14,15]

It should be noted that in aqueous buffer, the products of iron–artemisinin reaction were different from those obtained from the reaction performed in organic solvents. It was suggested elsewhere that the mode of action of artemisinin involved the cleavage of the endoperoxide bridge by iron, yielding a carbon centred free radical[2—4,13] which, in turn, alkylated the parasite-specific proteins.[5] It was also demonstrated that artemisinin and its derivatives have undergone a variety of unusual rearrangement reactions under different thermal, basic and acidic conditions.[2—4,6—12] In contrast, our study found that the major reaction product under an aqueous buffer condition was dihydroartemisinin which exhibited more potent antimalarial activity than artemisinin. This suggested that under physiological conditions, iron could react with artemisinin, giving active metabolites, the main product should be dihydroartemisinin, and probably oxygen free radicals during redox reaction of artemisinin. Thus, the antimalarial activity of the drug may be due to 1) the interaction of intact compound without chemical reaction, 2) the chemical reaction of the compound and/or its degradation products with the parasite biomolecules or 3) oxygen free radical occurring during redox reaction.

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