Binding Reaction of Hemin with Chloroquine, Quinine and Quinidine in Water-propylene Glycol Mixture

MAVAKALA, B. K.[a,b] NLANDU, B. B.[b] MPIANA, P. T.[b] GUSHIMANA, Z. Y.[b]
YU, Zhi-Wu*,[a](尉志武)
[a] *Ministry of Education Key Laboratory of Bioorganic Phosphorous Chemistry & Chemical Biology, Department of Chemistry, Tsinghua University, Beijing 100084, China*
[b] *Department of Chemistry, Biomacromolecules Laboratory, Kinshasa University, B.P. 190, Kinshasa, R. D. Congo*

The interaction of hemin with chloroquine, quinine and quinidine was investigated in 50% water-propylene glycol mixture at pH = 9, 8.1, 7.4 and 6.8 using a spectrophotometric method. The data could be well fitted into a model consistent with the formation of a 1:1 complex between the reacting partners. In addition, the results indicated that hemin complexed more strongly with quinidine than with chloroquine and quinine, and the binding constants were pH-dependent. Moreover, it was proved that the water-propylene glycol mixture is well suitable to the study of the systems containing hemin and quinoline-based drugs.

Keywords hemin, antimalarial drug, complex, pH-dependence, propylene glycol

Introduction

More than 40% of the world's population lives in areas where malaria is endemic and each year 300—400 millions cases of infections are recorded. In Africa, official estimations of annual mortality indicate that 1—3 millions cases of deaths are due to malaria. Most of the victims are children under 5 years of age.[1,2] With the ever-increasing multidrug resistance developed by *Plasmodium falciparum* (human malaria parasite),[3-5] antimalarial drugs are intensively investigated to understand their mode of action and the factors responsible of the resistance. A better understanding of the mode of action may provide a rational basis to improve the antimalarial activity of the existing drugs and to design new drugs (like artemisinin derivatives, trioxaquines).[6-10]

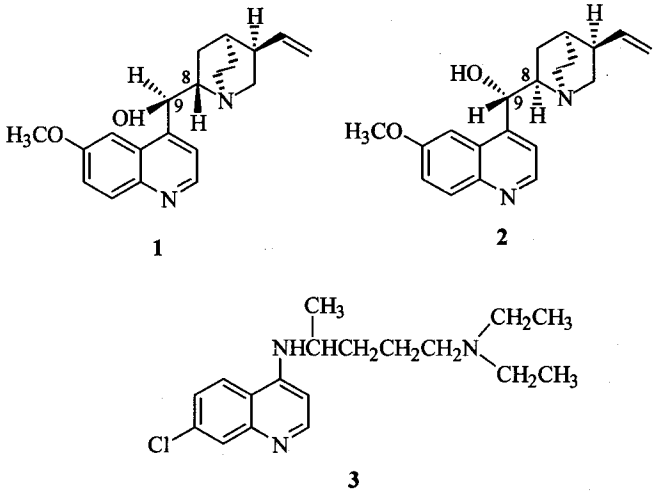
Unfortunately, the precise mode of action of antimalarial drugs remains uncertain. Frequently, controversial discussions in literatures are given.[11-13] The most interesting and developed theory suggests the binding of hemin (ferriprotoporphyrin IX) with these drugs as the prerequisite for their antimalarial action, leading to the formation of a complex.[3,12-19] Chou *et al*.[12] investigated the interaction between hemin and quinoline drug in aqueous medium. This medium, however, presents some limitations in regard to the solubility of the reacting partners. Hemin is soluble in alkaline aqueous solution, whereas quinoline compounds are soluble in acidic aqueous solution. A stable state of hemin in neutral or weakly acidic aqueous solution can not be achieved because of its tendency to dimerize. As a result, nonconsistent stoichiometries of hemin-quinoline complexes in aqueous solution, such as 2:1, 4:1 and 7:1 complexes, were reported.[20,21] Therefore, it is important to find a proper solvent within which dimerization of hemin can be prevented and, at the same time, both hemin and drugs are soluble. Effort has been made to solve the problem by using 40% aqueous DMSO to study the complexes between several quinoline antimalarials and monomeric hemin, and 1:1 stoichiometry was proposed.[22]

In our previous studies, the interaction of hemin with chloroquine and quinine and those of deuterohemin (hemin without vinyl groups) with quinine were investigated in 50% water-ethyleneglycol mixture.[23,24] In this medium, which is thermodynamically close to water,[25] the dimerization process of hemin could be well controlled and 1:1 stoichiometry of the complex was recorded.

Although this complex can be used for parasitologic assays *in vitro*, ethylene glycol is toxic for assays *in vivo*.[26] Another way to solve the problem is to find an alternative medium, which presents the same thermodynamic advantage as ethylene glycol-water mixture and, at the same time, is nontoxic. In this article, we report the results on the interaction of hemin with chloroquine, quinine and quinidine (Scheme 1) in 50% water-propylene glycol mixture at various pH values using a spectrophotometric titration method. The mixed solvent is of practical importance because the organic molecule is much less toxic than ethylene glycol and is commonly used as pharmaceutical adjuvant.

* E-mail: yuzhw@tsinghua.edu.cn
Received September 26, 2002; revised January 8, 2003; accepted March 10, 2003.
Project supported partially by the Tropical Disease Research/World Health Organization, the National Natural Science Foundation of China (No. 20133030) and the China Scholarship Council (No. 99180003).

Scheme 1 Structures of three quinoline-based drugs, quinine (**1**), quinidine (**2**) and chloroquine (**3**)



Experimental

Hemin and quinoline drug solutions

Hemin (Aldrich) stock solution, 306 $\mu mol \cdot L^{-1}$ in concentration, was prepared by dissolving 10 mg of it in 25 mL of alkaline distilled water, followed by addition of equivalent volume of propylene glycol (Sigma). Chloroquine (Sigma), quinine and quinidine (Merck) stock solutions were prepared by dissolving 51.6, 32.4 and 78.3 mg of them, respectively, in 25 mL of acidic distilled water, completed with addition of equivalent volume of propylene glycol. 0.1 $mol \cdot L^{-1}$ tris (hydroxymethyl)-methylamine (Aldrich) was used as buffer for all solutions.

pH *and optical absorption measurements*

The pH values were determined with Metrohm E 604 pH-meter equipped with a glass electrode. This electrode was kept soaked in 3 $mol \cdot L^{-1}$ KCl solution and calibrated with aqueous standard buffers. A Perkin Elmer Lambda 2 UV-Visible Spectrophotometer was used for titration. Temperature was controlled at (25.0 ± 0.1) ℃. The titration was carried out at the hemin characteristic Soret band at 396 nm by mixing a constant volume (0.31 mL) of hemin solution with various volumes of drug solutions, and then diluted to 5 mL. Thereby, hemin remains at constant concentration (19 $\mu mol \cdot L^{-1}$) while drugs concentrations are changed in the range of 0—1300 $\mu mol \cdot L^{-1}$.

Data analysis

It is assumed that the interaction between hemin (H) and antimalarial drug (Q) can be described according to the equilibrium shown below:

$$H + Q \overset{K}{\rightleftharpoons} HQ \qquad (1)$$

In diluted solutions, the association constant of complex K can be written as follows:

$$K = \frac{c_{HQ}}{c_H \cdot c_Q} \qquad (2)$$

and

$$c_H^0 = c_H + c_{HQ} \qquad (3)$$

$$c_Q^0 = c_Q + c_{HQ} \qquad (4)$$

where c_{HQ} is the concentration of complex and c_H^0 and c_Q^0 are the initial concentrations of hemin and drug, respectively.

The optical absorption of the system during titration can be written as:

$$E/d = c_H\varepsilon_H + c_{HQ}\varepsilon_{HQ} \qquad (5)$$

where E and d are the optical density and the light path, respectively. ε_H and ε_{HQ} are the molar extinction coefficients of hemin and its complex solutions.

Combining Eqs. (2), (3) and (4) with Eq. (5), the following equation is obtained:

$$E = E_0 + 1/2d\Delta\varepsilon[c_H^0 + c_Q^0 + 1/K - \sqrt{(c_H^0 + c_Q^0 + 1/K)^2 - 4c_H^0 \cdot c_Q^0}] \qquad (6)$$

where E_0 is the molar extinction of hemin solution at $c_Q^0 = 0$, and $\Delta\varepsilon$ ($\Delta\varepsilon = \varepsilon_{HQ} - \varepsilon_H$) is the difference of the molar extinction coefficients between hemin complex and free hemin. The basic data are initial concentrations of hemin (c_H^0) and drug (c_Q^0) and the corresponding optical extinction of hemin (E). With these data, parameters particularly the equilibrium constant K, can be fitted according to Eq. (6) with the help of Microsoft Origin 6.1 package.

Results and discussion

The spectrum range from 300 to 500 nm was selected to study the interaction of hemin with the quinoline-derived antimalarials. This is because the induced spectral modifications in the presence of the drugs are more significant in this range than in the remainder of the UV-Vis region. More specifically, the wavelength of 396 nm was selected to determine the constants of complexation because of the greatest variation of the optical density observed in the presence of the antimalarial drugs.

Titration of hemin by increasing amount of drugs in mixed water-propylene glycol solutions gives typical spectral changes as exemplified in Fig. 1. They are similar to those observed on deuterohemin-quinine, hemin-chloroquine and hemin-quinine interactions in other mediums.[23,24,27]

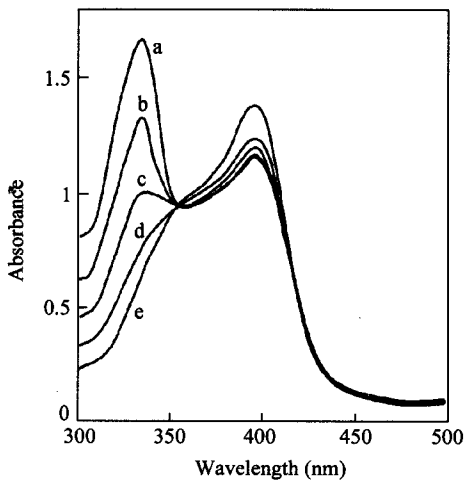


Fig. 1 Spectrophotometric titration curves of hemin (19 μmol·L^{-1}) by chloroquine solutions at concentrations of 1300 (a), 1000 (b), 550 (c), 40 (d) and 0 μmol·L^{-1}(e), with 50% water-propylene glycol mixture as solvent and 0.1 mol·L^{-1} tris(hydroxymethyl)methylamine as buffer (pH = 9).

The absorption band centered around 332 nm is from the quinoline derivative and that centered at 396 nm is from hemin. From Fig. 1 it can be seen that addition of chloroquine drug modifies markedly the hemin spectrum, but the peak maximums are still at about 396 nm. This indicates that the complexation does not involve significant modifications on the structure of the porphyrin ring of the ferriprotoporphyrin IX. Another feature that can be seen for all the three drugs is the appearance of an isosbetic point located at around 350 nm on the titration curves.

The experimental data were fitted into a 1∶1 complex model as described mathematically in Eq. (6). Selected such results with the total drug concentration as the only changing parameter are shown in Fig. 2. It can be seen

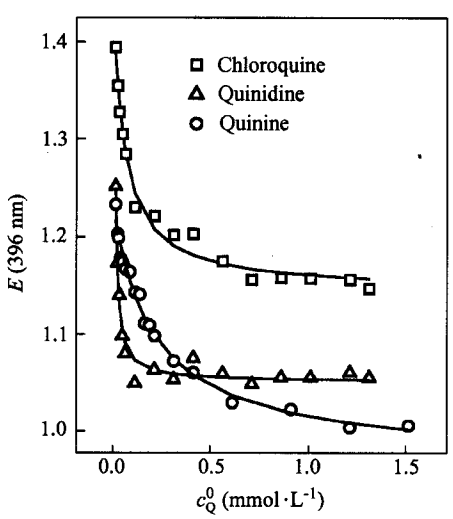


Fig. 2 Variation in absorbance of hemin at 396 nm as function of total concentration of the quinoline-based drugs (pH = 9).

that the extinction of the hemin solution decreases with increasing total drug concentration. This trend is consistent with previous results and can be attributed to complex formation between the drug and hemin.[23,24,27] The solid curves in the figure are fitted data with the experimental results according to Eq. (6). Correlation coefficients of the nonlinear fittings are better than 0.9, which implies that the titration curves can be well described by the 1∶1 complexation scheme. Similar variation in absorbance of hemin at 396 nm as function of total drug concentration has been obtained at other values of pH and the results are also consistent with the formation of 1∶1 complex. Values of binding constants at various pH obtained from these titration curves are summarized in Table 1. As highlighted by values of binding constants in Table 1, K values are in the same order of magnitude as those obtained in water-ethylene glycol mixture.[23,24]

In fact, the complexation of ferriprotoporphyrin IX with the drug is believed to play the role to bring back the hemin into solution in order to prevent it from polymerization. The ability of quinoline drug to complex with hemin will inhibit the formation of haemozoin (β-haematin) *in vivo*. The drug that has a greater affinity with hemin should maintain more hemin in solution and is thus more effective. This means that quinidine should have the highest efficiency, then chloroquine, and finally quinine, based on the data in Table 1. But in practical applications, an opposite trend is observed, probably due to the emergence of new resistant strains of malaria parasites against the existing and commonly used antimalarial drugs. As a matter of fact, in some areas (the case in D. R. Congo, for example) quinine appears more effective than chloroquine. This proves that the strength of haematin-quinoline interactions does not directly correlate with antiplasmodial activity. It indicates that haematin binding is a necessary, but not sufficient requirement for antiplasmodial activity.[28]

Table 1 Binding constant of hemin-drug complexes at various pH

pH	K (10^5 L·mol^{-1})		
	Hemin-chloroquine	Hemin-quinine	Hemin-quinidine
9.0	0.17 ± 0.03	0.05 ± 0.01	2.17 ± 0.43
8.1	0.22 ± 0.04	0.15 ± 0.03	4.17 ± 0.83
7.4	0.33 ± 0.06	0.11 ± 0.03	1.77 ± 0.94
6.8	0.40 ± 0.10	0.11 ± 0.02	2.87 ± 0.92

In regard to the molecular basis of the hemin-drug interactions, rather less is known about the structures of these complexes. In fact, the complexes between 4-aminoquinolines and hemin are almost certainly p-p complexes.[28] It means that there is an interaction between the aromatic ring of the quinoline and the porphyrin structure. In addition, hydrophobic interaction, electronic and steric factors also play important roles in influencing the structures of such complexes. Results from the present study show that chloroquine interacts more strongly with ferriprotoporphyrin IX than quinine does. It is suggested that the

flexible side aliphatic chain of the chloroquine structure, which is less crowded than that of the stiff quiniclidine group of the quinine structure, stabilizes hemin-chloroquine interaction. It is also supposed that a hydrogen-bonding interaction between the side-chain amine group of chloroquine and the heme propionate group may play a role in the hemin-chloroquine complex stability. In addition, the stability of these complexes is supported by computational results. A molecular mechanics study of the interaction between chloroquine and an iron-porphyrin model for *N*-acetylmicroperoxidase-8 revealed a minimum energy arrangement with coplanar interaction of the quinoline and iron-porphyrin ring, but could not define a preferred conformation for the complex.[29]

It is interesting to note that the conformation of drugs affects their affinity with hemin. As can be seen from Scheme 1, quinine differs from quinidine only at positions C-8 and C-9, the former has 8*S*,9*R* structure and the latter has 8*R*,9*S* structure.[30] The data showed significantly different affinity to hemin of the two chiral isomers.

Further more, it can also be seen that *K* values are pH-dependent. That dependence is probably due to acido-basic equilibrium influence on electrostatic interactions between hemin and the drugs. Due to their different pK_a values, reacting partners have different electric charge at different pH values.[23,27,31]

The results in the present study have paved a possible way to envisage the parasitologic assays *in vivo* with propylene glycol instead of ethylene glycol as solvent. With the help of the new solution system, no separation of the hemin complex from the solvent is needed and the working pH-values can be adjusted as required. The suggested medium could also be used to evaluate the antimalaric activity of complexes of hemin with other quinoline-based chemicals such as trioxaquines or with non quinoline drugs such as artemisinin and its derivatives like artesunate, artmether and dihydroartemisinin.

References

1 World Health Organization, Tropical Disease Research, *Thirteenth Programme Report of the UNDP (United Nations Development Programme)*, TDR Publication, Geneva, **1995—1996**, pp. 44—45.

2 Hoffman, S. L. *New England J. Med.* **1996**, *335*, 124.

3 Berman, P. A.; Adams, P. A. *Free Radical Biol. Med.* **1997**, *22*, 1283.

4 Hien, T. T.; White, N. J. *Lancet* **1993**, *341*, 603.

5 Wyler, D. J. *New England J. Med.* **1983**, *308*, 875.

6 Dechy-Cabaret, O.; Benoit-Vical, F.; Robert, A.; Meunier, B. *ChemBioChem* **2000**, *1*, 281.

7 Klayman, D. L. *Science* **1985**, *228*, 1049.

8 China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials *J. Trad. Chin. Med.* **1982**, *2*, 3.

9 Jiang, H. L.; Chen, K. X.; Tang, Y.; Chen, J. Z.; Ji, R. Y. *Chin. J. Chem.* **1995**, *13*, 131.

10 Gong, D. H.; Li, J. F.; Yuan, C. Y. *Chin. J. Chem.* **2001**, *19*, 1263.

11 Krogstad, D. J.; Schlesinger, P. H.; Gluzman, I. Y. *J. Cell Biol.* **1985**, *101*, 2302.

12 Chou, A. C.; Chevli, R.; Fitch,C. D. *Biochemistry* **1980**, *19*, 1543.

13 Egan, T. J.; Ross, D. C.; Adams, P. A. *S. Afr. J. Sci.* **1996**, *92*, 11.

14 Yayon, A.; Cabantchik, Z. I.; Ginsburg, H. *Proc. Natl. Acad. Sci. U. S. A.* **1985**, *82*, 2784.

15 Sugioka, Y.; Suzuki, M.; Sugioka, K.; Nakano, M. *FEBS Lett.* **1987**, *223*, 251.

16 Macomber, P. B. *Nature* **1967**, *214*, 937.

17 Zhang, Y.; Gosser, D. K.; Meshnick, S. R. *Biochemistry* **1992**, *43*, 1805.

18 Meshnick, S. R.; Jefford, C. W.; Posner, G. H.; Avery, M. A.; Peters, W. *Parasitol. Today* **1996**, *12*, 79.

19 Chen, Y.; Zhu, S. M.; Chen, H. Y. *J. Peking Univ. (Nat. Sci. Ed.)* **2001**, *37*, 255 (in Chinese).

20 Moreau, S.; Perly, B.; Biguet, B. *Biochimie* **1982**, *64*, 1015.

21 Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. *Biochem. Pharmacol.* **1998**, *55*, 727.

22 Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden J. *J. Med. Chem.* **2000**, *43*, 283.

23 Gushimana, Y.; Doepner, B.; Martinez, H. E.; Ilgenfritz, G. *Biophys. Chem.* **1993**, *47*, 153.

24 Gushimana, Z. Y.; Mpiana, P. T.; Tshilanda, D. D. *Ann. Fac. Sci. Kinshasa* **1996**, *2*, 145.

25 Feustel, D. *M.S. Thesis*, Koln University, Koln, **1984**.

26 Haley, T. J.; Berndt, W. O. *Handbook of Toxicology*, Harper & Row Publisher, New York, **1987**.

27 Constantinidis, I.; Satterlee, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 927.

28 Egan, T. J.; Ross, D. C.; Adams, P. A. *FEBS Lett.* **1994**, *352*, 54.

29 Marques, H. M.; Vester, K.; Egan, T. J. *J. Inorg. Biochem.* **1996**, *64*, 7.

30 Ribeiro, M. C. D.; Augusto, O.; Ferreira, A. M. D. *J. Inorg. Biochem.* **1997**, *65*, 15.

31 Kuhn, S. *Ph.D. Dissertation*, Koln University, Koln, **1995**.

(E0209262 LI, L. T.; LING, J.)