Biochemical Pharmacology, Vol. 36, No. 8, pp. 1267–1273, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

LYSIS OF MALARIAL PARASITES AND ERYTHROCYTES BY FERRIPROTOPORPHYRIN IX–CHLOROQUINE AND THE INHIBITION OF THIS EFFECT BY PROTEINS

YA ZHANG and ERNST HEMPELMANN

Institut für Biochemie II, Universität Heidelberg, Im Neuenheimer Feld 328, D-6900 Heidelberg, Federal Republic of Germany

(*Received* 12 *August* 1986; *accepted* 20 *October* 1986)

Abstract—Ferriprotoporphyrin IX(FP) lysed both erythrocytes and isolated *Plasmodium falciparum* as judged by decrease in turbidity of erythrocyte and parasite suspensions. The lytic effect of FP on erythrocytes was enhanced by chloroquine(CQ). In the presence of 2.5–20 μM CQ, 5 μM FP led to complete hemolysis within 45 min. However, the lytic effect of FP or FP–CQ on both erythrocytes and parasites was inhibited completely by proteins. The protein inhibition was non-specific.

This finding, the failure of FP and FP–CQ to cause hemolysis and lysis of malarial parasites in a protein-containing medium, does not support the "FP–CQ complex hypothesis" for the antimalarial action of chloroquine.

Many studies have demonstrated that the antimalarial drug, chloroquine, accumulates in erythrocytes infected with malarial parasites [1–5]. Some hypotheses have been proposed to explain the accumulation of the drug in the parasites and its antimalarial action [6–9].

In 1964, two groups observed simultaneously that chloroquine could form a complex with ferrihemic acid, which is released during hemoglobin digestion by malarial parasites [10, 11]. The complex formation was considered to be related to the antimalarial action of chloroquine. Further studies from other laboratories indicated that ferriprotoporphyrin IX (FP)* could bind specifically to the chloroquine to form a FP–chloroquine complex [12–15]. The complex is toxic for biological membranes and results in the lysis of the malarial parasites [16–19]. Fitch *et al.* proposed a mode of antimalarial action of chloroquine [20–22], postulating that FP, formed during hemoglobin degradation by malarial parasites, served as a chloroquine receptor [14, 15, 23]. Binding of chloroquine to FP would divert FP from incorporation into non-toxic substances (e.g. malarial pigment) to form a toxic FP-chloroquine complex [23–25].

However, all experiments in support of the FP–chloroquine complex hypothesis were carried out in the absence of proteins [14, 16, 23, 24]; significantly the lytic effect of FP and FP–chloroquine complex on biological membranes was inhibited by bovine serum albumin (BSA) [25]. Our previous results have shown that the antimalarial action of chloroquine is not correlated with the presence of malarial pigment in the parasites [26] and that chloroquine fails to form a FP–chloroquine complex with FP in protein-containing solution.†

In the present report, we describe the lytic effect of FP and of the FP–chloroquine complex on erythrocytes and isolated malarial parasites as well as the inhibition of the effect by proteins.

MATERIALS AND METHODS

Hemolytic effect of FP and FP–CQ on human erythrocytes and the inhibition of the hemolysis by proteins. Erythrocytes from healthy A(+) donors were collected in acid citrose dextrose solution [27]. The erythrocytes were washed by suspension in 10 vol. of isotonic medium [24] and centrifuged at 1500 *g* min at 10°. After three washes, the erythrocytes were resuspended in the same medium for hemolysis experiments.

Hemolysis was monitored by measuring the changes in turbidity of these suspensions at 700 nm with a Schimatzu UV-180 photometer [19, 24]. A 3 ml cuvette (optical path: 10 mm) was filled with a suspension of washed erythrocytes and preincubated at 37° for 15 min before addition of FP, CQ and protein solutions. The suspension for each measurement was adjusted to an absorbance of about 0.65 (approximately 5% erythrocytes). All reference tubes had the same composition as the corresponding test tubes but contained no erythrocytes.

Chloroquine diphosphate and FP chloride (hemin) were from Sigma Chemical Co (St. Louis, MO), BSA was obtained from Serva Feinbiochemica (Heidelberg) and hemoglobin and lysozyme were purchased from Merck (Darmstadt).

To avoid problems associated with the polymerization of FP in water [28, 29], a fresh hemin solution had to be used in all experiments. As stock solution, 1 mM or 5 mM hemin was prepared in double-distilled water after first dissolving it in a minimal amount of 0.1 N NaOH and putting it in an ultrasonic bath for 5 min. The stock hemin solution was stored at 4° for 2 hr. CQ stock solution (1 mM) and protein stock solution were prepared with isotonic medium. In measurements of hemolytic response to any two components or more among FP,

* Abbreviations used: FP, ferriprotoporphyrin IX; CQ, chloroquine; BSA, bovine serum albumin.

† Y. Zhang *et al.*, submitted.

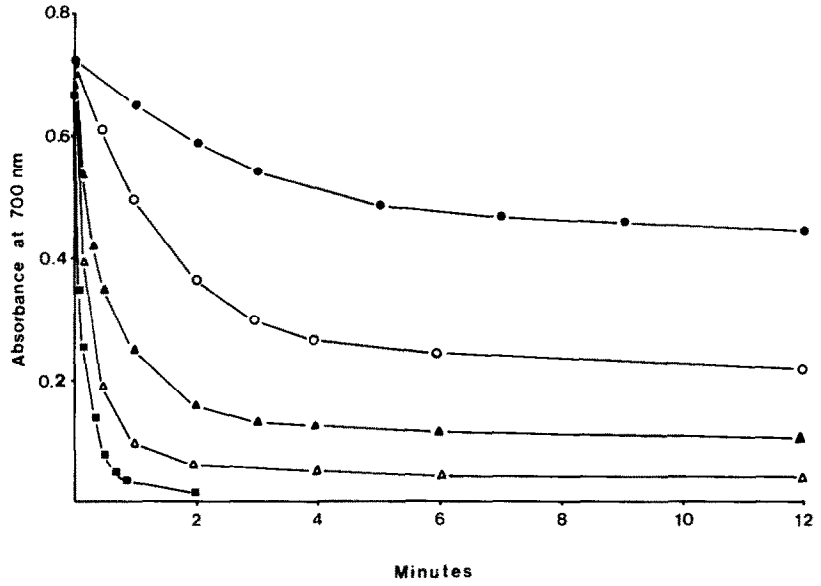

Fig. 1. Time-course of FP-induced hemolysis. A 0.5% suspension of washed erythrocytes was incubated in the standard medium with 5 μM (●), 10 μM (○), 15 μM (▲), 20 μM (△) and 30 μM (■) for the indicated time intervals at 37° and pH 7.4 in the dark.

CQ and proteins, the components were preincubated at 37° for 15 min to insure complete interaction. All incubations of suspension with FP were carried out in the dark.

Lytic effect of FP–CQ on isolated Plasmodium falciparum and the inhibition of this effect by proteins. *Plasmodium falciparam* (FCB-strain) was used in the experiments. The parasites were cultured in medium RPMI-1640 (GIBCO Limited, Scotland), supplemented with 25 mM HEPES, 32 mM $NaHCO_3$, 50 mg/l hypoxanthine and 10% (v/v) human serum from A(+) blood according to the candle jar method [30].

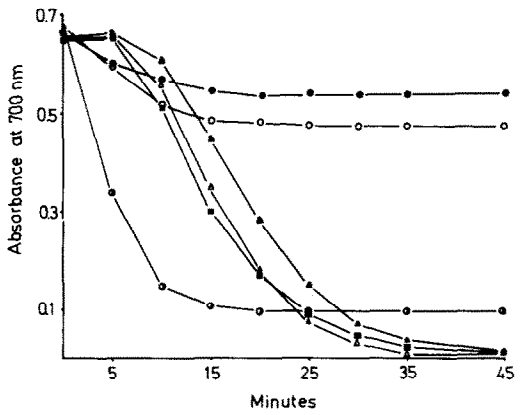

Fig. 2. Concentration effect of chloroquine on enhancement of FP-induced hemolysis. FP solution preincubated with various concentrations of chloroquine was added to the suspension of erythrocytes. The changes of absorbance were recorded at 700 nm: ●, 5 μM FP; ○, 5 μM FP plus 0.2 μM chloroquine; ◑, 5 μM FP plus 1 μM chloroquine; ■, 5 μM FP plus 2.5 μM chloroquine; ▲, 5 μM FP plus 5 μM chloroquine; △, 5 μM plus 20 μM chloruine. In the absence of FP, chloroquine causes no hemolysis.

The parasites were synchronized by 5% sorbitol for 10 min at room temperature [31]. The resulting infected erythrocytes were washed twice with the medium and then added to washed fresh erythrocytes to maintain a parasitemia of 1.5% and the cultures were re-established. After 48 hr (one life cycle of the parasite), the parasites were culture with a 2% suspension of erythrocytes with twice daily medium changes [32].

At about 30 hr in the second cycle of the synchronous parasites, the infected erythrocytes at the trophozoite stage, with about 30% parasitemia, were collected by centrifugation at 1500 *g* for 5 min at 10°. The erythrocytes were washed in isotonic medium and then lysed by incubation at 37° for 10 min in 20 volumes of 15 mg% saponin (Sigma, St Louis) in isotonic medium [16]. After low speed centrifugation the pellet, consisting of the parasites, was retained and the supernatant, consisting of soluble material and erythrocyte ghost, was discarded. The parasites were washed twice and resuspended at a density of approximately 10^7 per ml in isotonic medium for measuring the lytic effect of FP–CQ within 2 hr. The lytic effect was monitored by the method as described above the erythrocytes.

RESULTS

Hemolytic effect of FP and FP–CQ on human erythrocytes

In this study, carried out with fresh blood cells, less than 1% hemolysis occurred in controls without FP during the incubation interval from 30 to 90 min at 37°. We found that the absorbance at 700 nm in a range up to 0.65 was an approximate function of erythrocyte concentration so that hemolysis can be expressed by its reduction. Nevertheless, all calculations concerning hemolysis percentage were

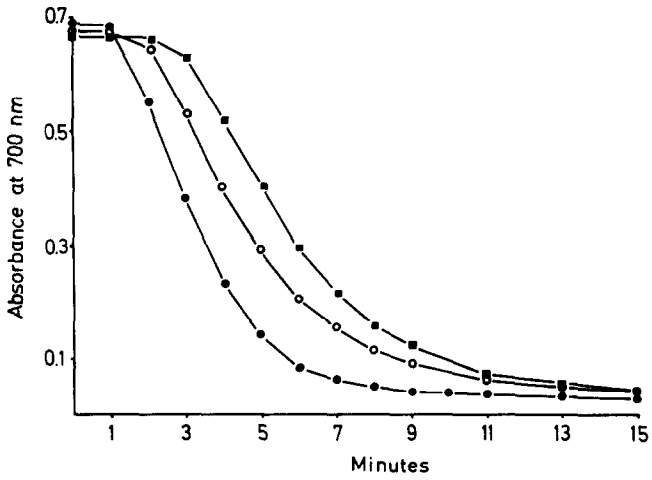

Fig. 3. Time-course of hemolysis by FP–chloroquine at high concentration. Preincubated FP–chloroquine solution with high concentration was added to the suspension of erythrocytes. The changes of absorbance were recorded at 700 nm: ■, 20 μM FP plus 20 μM chloroquine; ○, 40 μM FP plus 40 μM chloroquine; ●, 80 μM FP plus 80 μM chloroquine.

adjusted by means of an erythrocyte suspension–absorbance standard curve.

Similar studies have been described in the past [33–35] but our study showed some new results.

The time-course of hemolysis in the presence of various concentrations of FP indicated that the degree and the rate of hemolysis depended upon FP concentration (Fig. 1). AT FP concentrations greater than 15 μM, 50% hemolysis of the suspension occurred within 30 sec. The hemolysis by FP was rapid but seemed incomplete, particularly at low concentrations. 5 μM FP did not show a clear lytic effect within 45 min (Fig. 1).

The time-course of hemolysis by FP–CQ showed some differences from that by FP alone.

1. Compared to only about 20% hemolysis produced by 5 μM FP alone, 5 μM FP with 2.5–20 μM CQ caused 100% hemolysis within 45 min and had a similar time-course (Fig. 2). This suggests that CQ enhances the FP hemolytic effect and that the enhancement of FP hemolysis by CQ is independent of the CQ concentration in the indicated concentration range.

2. In contrast to FP, hemolysis by FP–CQ did not occur immediately but showed a lag phase. The time-course of hemolysis appeared as a sigmoidal curve (Fig. 2). In incubation with 5 μM FP alone for 45 min, 50% hemolysis of total erythrocytes occurred within the first 5 min while no hemolysis was seen during the initial period in the suspensions of erythrocytes incubated with 5 μM FP in combinations with from 2.5 to 20 μM CQ (Fig. 2). With higher concentrations of FP, and FP with an equal amount of CQ, hemolysis by FP alone was much more rapid than that by FP–CQ (Figs 1 and 3). The time of 50% maximum hemolysis by FP was a few seconds but that by FP–

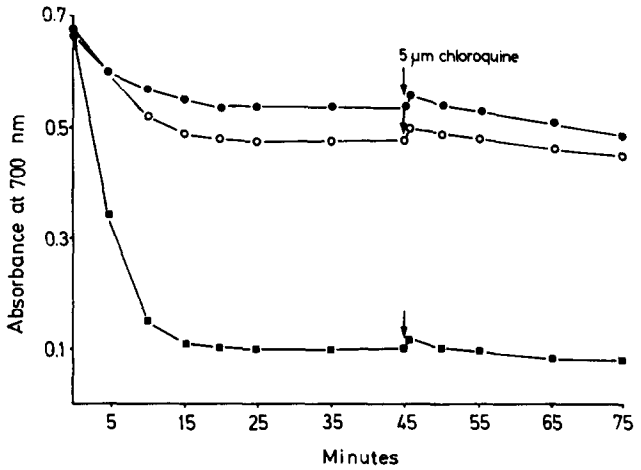

Fig. 4. Effect of chloroquine on the preincubation of FP with erythrocytes. A 0.5% suspension of washed erythrocytes was incubated for 45 min under the condition described for Fig. 1: ●, 5 μM FP; ○, 5 μM FP plus 0.2 μM chloroquine; ■, 5 μM FP plus 1 μM chloroquine. Another 5 μM chloroquine was added to every group after the incubation for 45 min.

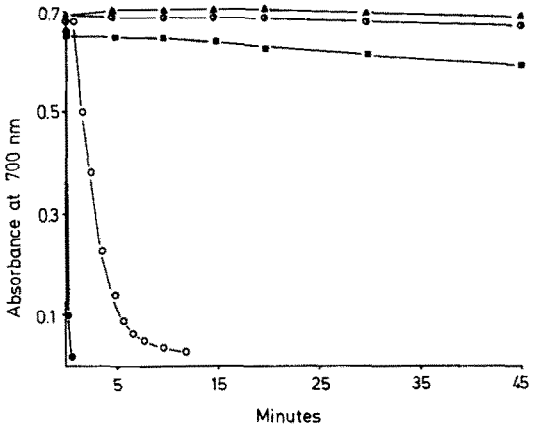

Fig. 5. Inhibition of FP- and FP–chloroquine-induced hemolysis by BSA. FP or preincubated FP–chloroquine, FP–BSA and FP–chloroquine–BSA were added to the suspension of erythrocytes. The changes of absorbance were recorded at 700 nm under the condition of incubation described for Fig. 1: ●, 20 μM FP; ○, 80 μM FP plus 80 μM chloroquine; ▲, 80 μM FP plus 1% BSA; ◑, 80 μM FP and 80 μM chloroquine plus 1% BSA; ■, 80 μM FP and 80 μM chloroquine plus 0.1% BSA.

CQ was several minutes even if the concentration of FP–CQ (80 + 80 μM) was higher than that of FP alone (10–30 μM).

3. If a lower concentration of CQ (lower than 1 μM) was added to the 5 μM FP system, the hemolytic effect was enhanced partially. 0.2 or 1 μM CQ increased hemolysis by 5 μM FP from 18.2% to 28.4 or 85% in 45 min. However, the effect occurred immediately and the lag phase of the time-course disappeared, which was similar to the hemolytic effect of FP alone (Figs 1 and 2).

4. Suspensions of erythrocytes were preincubated with 5 μM FP or FP with the low concentration of CQ for 45 min, to obtain partial hemolysis. To these suspensions, another 5 μM CQ (i.e. enough to enhance the hemolytic effect by FP) (Fig. 2), was added, but further hemolysis was not observed within another 30 min (Fig. 4).

Inhibition of hemolytic effect of FP and FP–CQ by proteins

The above results have shown hemolytic effect of FP and FP–CQ. However, these experiments were carried out under protein-free condition. When 0.1% (15 M) BSA was added to the system, the hemolytic effect of FP or FP-CQ was prevented even though the concentration of FP or FP–CQ in the incubate was 15 times higher than the hemolytic concentration (Fig. 5).

The concentration effect of various proteins on the inhibition of hemolysis by FP–CQ shows that BSA, hemoglobin and lysozyme are capable of inhibiting the hemolysis by FP–CQ to almost the same degree (Fig. 6). Almost total inhibition is produced by all proteins at 1 mg/ml; a clear hemolysis was seen only in the solution containing less than 0.03 mg/ml proteins (Fig. 6).

Lytic effect of FP and FP–CQ on Plasmodium falciparum *and the inhibition of this effect by proteins*

Figure 7 shows that CQ alone (from 10 to 250 μM) does not cause a decrease in the turbidity of the parasite suspension, but FP alone (20 or 40 μM) leads to a decrease in the turbidity, due to lysis of the parasites [16]. However, FP or FP–CQ only reduced, but could not eliminate the turbidity of the parasite suspension which may be due to the presence of cell components which cannot be lysed by FP and do not

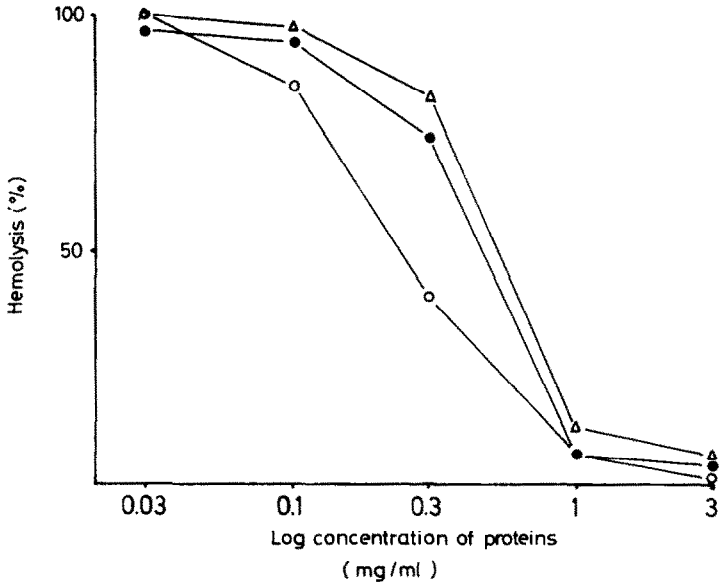

Fig. 6. Concentration effect of various proteins on the inhibition of hemolysis by FP–chloroquine. FP and chloroquine were preincubated with BSA, hemoglobin or lysozyme at various concentrations for 15 min at 37°. Suspension of erythrocytes was added to these preincubated solutions and then, incubated for 45 min at 37°. All groups contained 40 μM FP and 50 μM chloroquine, and with BSA (●), hemoglobin (○) and lysozyme (△) at various concentrations.

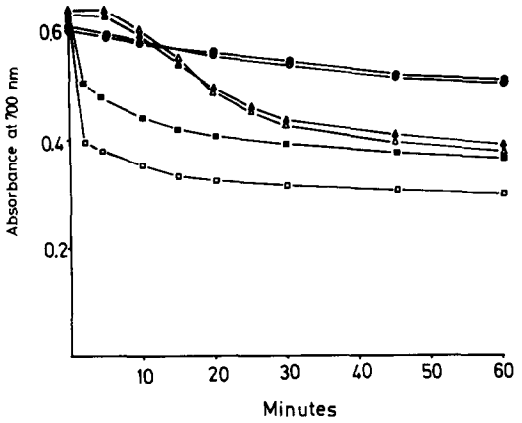

Fig. 7. Lytic effect of FP and FP–chloroquine on *Plasmodium falciparum*. A suspension with about 10^7 parasites per ml was incubated in the standard medium without FP (●), with 250 μM chloroquine (○), 20 μM FP (■), 40 μM FP (□), 20 μM FP with 20 μM chloroquine (▲) and 40 μM FP with 40 μM chloroquine (△) for the indicated time intervals at 37° and pH 7.4 in the dark.

exist in erythrocytes, e.g. parasite nuclear material and malarial pigment.

The lytic effect of FP–CQ on malarial parasites was relatively slow and its time-course was sigmoid and had a lag phase (Fig. 7). However, CQ did not enhance the lytic effect of FP on the malarial parasites (Fig. 7).

The lytic effect of FP and FP–CQ on the *Plasmodium falciparum* parasite was also blocked completely by BSA and hemoglobin (Fig. 8).

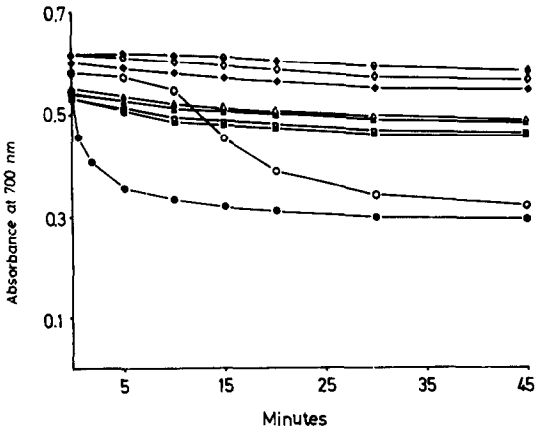

Fig. 8. Inhibition of lytic effect of FP and FP–chloroquine on *P. falciparum* by proteins. A suspension of isolated parasites was incubated under the condition described for Fig. 7. The concentration of FP and chloroquine, used in the experiment, is 40 μM respectively and the concentration of proteins is 0.5%: ●, FP alone; ○, FP with chloroquine; ■, BSA alone; △, BSA with FP; ▲, BSA with FP and chloroquine; ◆, hemoglobin alone; ◇, hemoglobin with FP; ◈, hemoglobin with FP and chloroquine; □, control group (without proteins, FP and chloroquine).

DISCUSSION

Our results confirm the observation of others that FP might serve as a hemolytic agent [33–35] and be toxic for the membranes of the malarial parasite [16, 19]. However, our finding of the difference in hemolytic time-course between FP and FP–CQ complex is in contrast to the result of Chou and Fitch [33], who suggested that CQ enhanced the hemolytic effect of FP by eliminating the lag phase. We found that the rate of hemolysis by FP–CQ is slower than that by FP alone although the degree of hemolysis was enhanced by CQ (Figs 1–3). This result suggests that the process of hemolysis by FP–CQ complex may not be the same as that by FP alone.

A similar difference in lytic effect of FP and FP–CQ complex was observed in the experiment with isolated *P. falciparum* (Fig. 7).

A more significant observation in the study is that the lytic effect of FP and FP–CQ on erythrocytes and malarial parasites is inhibited by proteins (Figs 5 and 8). The inhibitory effect of proteins is non-specific, as not only BSA but also hemoglobin and lysozyme can prevent the lytic effect of FP and FP–CQ (Figs 5 and 8). This result is in disagreement with the mode of the antimalarial action of CQ postulated by Fitch *et al.*, which suggests that as FP can bind to CQ, it can be regarded as a receptor for CQ, resulting in accumulation of the drug in erythrocytes infected with malarial parasites [14, 23, 36, 37]. The FP–CQ hypothesis states that as soon as CQ binding to FP, FP is diverted from incorporation into non-toxic substances to form a toxic FP–CQ complex, which can lead to lysis of the parasites [23–25]. However, an important factor appears to have been neglected in these studies as the experiments about FP–CQ binding and FP–CQ lysis were carried out under protein-free condition.

Many studies have shown that FP binds tightly to various proteins to form a non-toxic FP–protein complex and does not exist as free-FP under physiological conditions [38–41]. These results are in agreement with the fact that "free-FP", as a receptor of CQ hypothesized in the FP–CQ theory, could not be demonstrated in malarial parasites by experiments [42–44].

The behavior of CQ under physiological conditions is different from that of FP. Only one third of total CQ is bound to serum proteins [45, 46,*]. Other experiments indicate that CQ alone, at any concentrations, could not cause the lysis of the parasite membranes [16, 47–49]; suggesting that the antimalarial action of the drug is not due to the formation of a lytic FP–CQ complex. Our previous study showed that the antimalarial effect of CQ was not correlated with the presence of malarial pigment and FP in the parasites [26] and our recent experiments showed that FP with CQ formed a complex but this complex was not very stable. As soon as protein was added to the FP–CQ solution, the complex was dissociated and a new FP–protein complex was formed. The FP–protein complex possesses much higher stability than the FP–CQ complex* and is not toxic for biological membranes (Figs 5, 6 and 8).

Free-FP in water solution is very unstable and forms polymers rapidly [28, 29, 50]. It can bind to

proteins released from lysed biological membranes. This property may explain why FP alone, particularly at lower concentrations, produced only partial hemolysis in the early phase of incubation (Fig. 1). The partial hemolysis was not enhanced by adding another 5 μM CQ (Fig. 4), suggesting that there was no toxic free-FP after 45 min in the system, i.e. that FP had bound to components from lysed erythrocytes. CQ enhances the stability of FP by means of FP–CQ complex formation* and thus enhances the hemolytic effect of FP (Fig. 2).

Proteins at a concentration of 0.1%, which is much lower than protein concentration in cells, showed a strong inhibitory effect on the lysis by FP–CQ at a concentration of 40–80 μM, which is much higher than levels encountered by the parasites. This result suggests that the antimalarial action of CQ can not be fully explained by the FP–CQ hypothesis. We have demonstrated that CQ can not form an FP–CQ complex under physiological condition* and that neither FP nor FP–CQ can lead to lysis of erythrocytes and malarial parasites in protein solution (Figs 5, 6 and 8). For these reasons we feel that FP–CQ complex formation as a basis for the antimalarial action of CQ is less likely than some other mechanisms which have been proposed [51–55].

Acknowledgements—Our work is supported by the Deutsche Forschungsgemeinschaft (Schi 102/7-3).

Zhang is scholar of DAAD (Deutscher Akademischer Austausch-Dienst).

This work is part of Z. Y.'s PhD Thesis supervised by Professor D. Mecke at Tübingen University.

REFERENCES

1. O. R. Ladda and H. Sprinz, *Proc. Soc. exp. Biol. Med.* **130**, 524 (1969).
2. P. R. Macomber, R. L. O'Brien and F. E. Hahn, *Science* **152**, 1374 (1966).
3. W. Sirawaraporn, B. Panijpan and Y. Yuthavong, *Exp. Parasit.* **54**, 260 (1982).
4. Y. Yuthavong, *Life Sci.* **26**, 1899 (1980).
5. F. Verdier, J. LeBras, F. Clavier, I. Hatin and M. C. Blayo, *Antimicrob. Agents Chemother.* **27**, 561 (1985).
6. S. C. Chou, K. A. Conklin, M. R. Levy and C. D. Warhurst, in *Antimalarial drug. Handb. Exp. Pharm.* Vol 68/I, p. 281. Springer, Berlin (1984).
7. F. E. Hahn, *Antibiotics*, Vol. III (Eds. J. W. Ovcoran and F. E. Hahn), p. 58. Springer, Berlin (1974).
8. D. L. Looker, J. J. Marr and R. L. Stotish, in *Chemotherapy of Parasitic Disease* (Eds. W. C. Campbell *et al.*), p. 193. Plenum Press, New York (1986).
9. C. DeDuve, T. DeBarsy, B. Pool, A. Trouet, P. Tulkeus and F. Van Hoof, *Biochem. Pharmac.* **23**, 2495 (1974).
10. S. N. Cohen, K. O. Phifer and K. L. Yielding, *Nature, Lond.* **202**, 805 (1964).
11. F. W. Schueler and W. F. Cantrell, *J. Pharm. exp. Ther.* **143**, 278 (1964).
12. D. Balasubramanian and C. M. Rao, *Science* **223**, 828 (1985).
13. H. Blauer and H. Ginsburg, *Biochem. Int.* **5**, 519 (1982).
14. A. C. Chou, C. Chevli and C. D. Fitch, *Biochemistry* **19**, 1143 (1980).
15. A. Jearnpipatkul, P. Govitrapong, Y. Yuthavong, P. Wilairat and B. Panijpan, *Experimentia* **36**, 1063 (1980).
16. C. D. Fitch, R. Chevli, A. B. Banyal, G. Phillips, M. A. Pfallar and D. J. Krogstad, *Antimicrob. Agents Chemther.* **21**, 819 (1982).
17. C. D. Fitch, R. Chevli, P. Kanjananggulpan, P. Dutta, K. Chevli and A. C. Chou, *Blood* **62**, 1165 (1983).
18. H. Ginsburg and R. A. Demel, *Biochim. biophys. Acta* **723**, 316 (1983).
19. A. V. Orjih, H. S. Banyal and C. D. Fitch, *Science* **214**, 667 (1981).
20. C. D. Fitch, in *Malaria and the Red Cell*, Ciba Found. Symp. p. 222. Pitman, London (1983).
21. C. D. Fitch, P. Dutta, P. Kanjananggulpan and R. Chevli, in *Malaria and the Red Cell. Prog. in Clin. and Biol. Res.*, Vol. 155, p. 119. Liss, New York (1984).
22. E. W. McChesney and C. D. Fitch, in *Antimalarial Drug* II. *Handb. Exp. Pharm.*, Vol. 68/II, p. 3. Springer, Berlin (1984).
23. C. D. Fitch and R. Chevli, *Antimicrob. Agents Chemother.* **19**, 589 (1981).
24. H. S. Banyal and C. D. Fitch, *Life Sci.* **31**, 1141 (1982).
25. P. Dutta and C. D. Fitch, *J. Pharmac. exp. Ther.* **225**, 729 (1983).
26. Y. Zhang, K. S. O. Asante and A. Jung, *J. Parasit.* in press.
27. C. T. Capps and J. B. Jensen, *J. Parasit.* **69**, 158 (1983).
28. S. B. Brown, J. C. Dean and P. Johes, *Biochem. J.* **117**, 733 (1970).
29. Y. Inada and K. Shibata, *Biochem. biophys. Res. Commun.* **9**, 323 (1962).
30. J. B. Jensen and W. Trager, *J. Parasit.* **63**, 883 (1977).
31. C. Lambros and J. P. Vandenberg, *J. Parasit.* **65**, 418 (1979).
32. C. Raventos. Suarez, *In Vitro* **21**, 161 (1985).
33. A. C. Chou and C. D. Fitch, *J. clin. Invest.* **66**, 856 (1980).
34. A. C. Chou and C. D. Fitch, *J. clin. Invest.* **68**, 672 (1981).
35. I. Kirschner-Zlber, E. Rabizadeh and N. Shaklai, *Biochim. biophys. Acta* **690**, 20 (1982).
36. C. D. Fitch, *Proc. natn. Acad. Sci. U.S.A.* **54**, 521 (1969).
37. C. D. Fitch, *Science* **169**, 289 (1970).
38. H. F. Bunn and J. H. Jandl, *J. biol. Chem.* **243**, 465 (1968).
39. C. C. Liang, *Biochem. J.* **66**, 552 (1957).
40. A. C. Maehly, *Nature, Lond.* **192**, 630 (1961).
41. M. Rosenfeld and D. M. Surgenor, *J. biol. Chem.* **183**, 663 (1950).
42. D. C. Warhurst and D. J. Hockley, *Nature, London.* **214**, 936 (1967).
43. K. A. Yamada and I. W. Sherman, *Exp. Parasit.* **48**, 61 (1979).
44. A. Yayon, Z. I. Cabantchik and H. Ginsburg, *Proc. natn. Acad. Sci. U.S.A.* **82**, 2784 (1985).
45. S. A. Adelusi and L. B. Salako, *Br. J. clin. Pharm.* **13**, 451 (1982).
46. S. F. Parker and J. L. Irvin, *J. biol. Chem.* **199**, 889 (1952).
47. A. Yayon and H. Ginsburg, *Cell. Biol. Int. Rep.* **11**, 895 (1983).
48. A. Yayon, R. Timberg, S. Friedman and H. Ginsburg, *J. Protozool.* **31**, 367 (1984).
49. F. Wundlich, H. Stübig and E. Königk, *Tropenmad. Parasit.* **32**, 77 (1981).
50. G. H. Beaven, S. H. Chen, A. D'Albis Albis and B. Gratzer, *Eur. J. Biochem.* **41**, 539 (1974).
51. C. A. Homewood, C. D. Warhurst, W. Peters and V. C. Baggalley, *Nature, Lond.* **235**, 50 (1972).

* Y. Zhang *et al.*, submitted.

52. D. J. Krogstad and P. H. Schlesinger, *Biochem. Pharmac.* **35**, 547 (1986).
53. D. J. Krogstad, P. H. Schlesinger and I. Y. Gluzman, *J. Cell Biol.* **101**, 2302 (1985).
54. A. Yayon, Z. I. Cabantchik and H. Ginsburg, *Proc. natn. Acad. Sci. U.S.A.* **82**, 2784 (1985).
55. A. Yayon, Z. I. Cabantchik and H. Ginsburg, *EMBO J.* **3**, 2695 (1984).