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Synthesis and Optical Properties of the Chloroquine Enantiomers and Their Complexes With Ferriprotoporphyrin IX in Aqueous Solution

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ABSTRACT Chloroquine (CQ) enantiomers were prepared by a novel synthesis starting from either (S)- or (R)-pyroglutamic acid. Light-absorption spectra of CQ and of complexes of ferriprotoporphyrin IX (FP) with CQ were measured in dilute aqueous solutions at pH 7.3 and 11.3. Spectrophotometric titrations at these pH values indicated a mole ratio of FP:CQ of 2:1 for the FP-CQ aggregated complexes. Aqueous solutions of each of the CQ enantiomers (pH 7.3) and of their complexes with FP (pH 11.3) were investigated by circular dichroism (CD). At pH 11.3, the complexes of the two enantiomers showed CD-band extrema of opposite sign at 409–410 nm. CD-titrations at pH 11.3 confirmed a predominant mole ratio of FP:CQ of 2:1 in the complex. The possible origin of the optical activity of the FP-CQ complexes is discussed. *Chirality 10:556–563, 1998.*

KEY WORDS: chloroquine; enantiomers; synthesis; pyroglutamic acid; ferriprotoporphyrin IX; circular dichroism

In previous publications, the circular dichroism (CD) spectra of complexes of chiral antimalarial drugs such as (–)-quinine or (+)-quinidine with ferriprotoporphyrin IX (FP) have been described in detail.[1] It will be shown below that each of the chloroquine (CQ) enantiomers also forms optically active complexes with FP under certain conditions; however, the mechanism for the generation of optical activity differs from that observed in the previous cases.

Both CQ-stereoisomers can be obtained by optical resolution of novoldiamine, a synthetic intermediate, according to Blaschke et al.[2] Craig and coworkers reported the synthesis of (–)-(R)-CQ using L-glutamic acid as starting compound. However, slight racemization occurred during their reaction sequence.[3] The CQ-enantiomers investigated in the present work were prepared following our own synthetic route starting from (S)- and (R)-pyroglutamic acid, respectively. The optical purity of the products is in accordance with the data reported by Blaschke et al.[2]

The synthetic enantiomers of CQ were used in the present work for complex formation with FP, which exhibited significant CD bands in the FP-Soret region near 400 nm at alkaline pH values. Previous[4] and present light-absorption and CD data of the FP-CQ system also confirm complex formation under different conditions; FP-drug complexes have been considered to be of relevance in the mechanism of antimalarial drug action (see, for example, Chou et al.[5] and references cited therein). In Scheme 1, the structures of FP and of the enantiomeric forms of CQ are presented.

MATERIALS AND METHODS

Synthesis of Optically Active Chloroquine Diphosphate

The 10-step sequence described below starts from (S)-pyroglutamic acid **(1a)** (Scheme 2) and proceeds similarly with the (R)-antipode **(1b).** The intermediate (R)-5-methylpyrrolidone **(2a)** was prepared according to the literature.[6] Benzoylation to the imide **3a** made possible a ring opening of the lactam moiety with diethylamine to give diamide **4a.**[7] After reduction to diamine **5a** using complex hydrides, the benzylic group was removed by hydrogenolysis to yield (R)-4-amino-1-diethylaminopentane (novoldiamine),[2] which could be converted to the (–)-(R)-CQ-diphosphate by known procedures.[3,8,9]

Experimental Procedures

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. Solvents and common reagents were obtained commercially and used as received; dry THF was obtained by refluxing over sodium; 1,2-dichloroethane was stored over molecular sieve 4 Å. NMR spectra were determined on a Bruker (Karlsruhe, Germany) AC 80 or a Varian (Palo Alto, CA) Unity 300 plus spectrometer and were measured in $CDCl_3$. ^1H spectra refer to tetramethylsilane as internal standard, ^{13}C spectra to $CDCl_3$. IR spectra were

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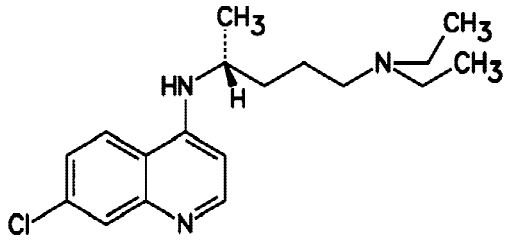
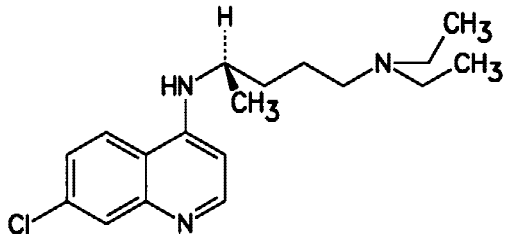


(–)-(R)-CQ



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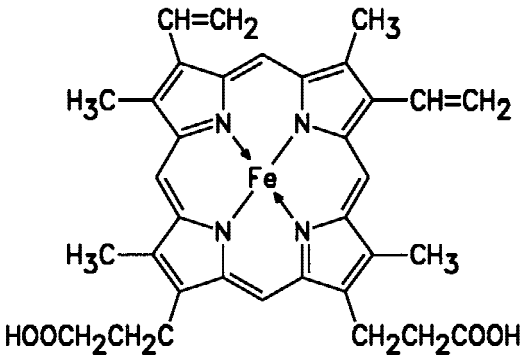


FP

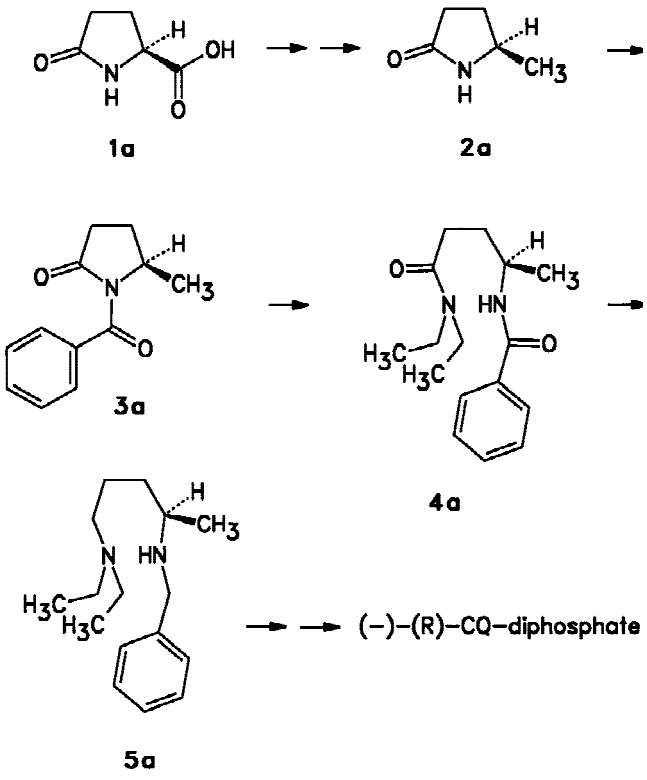
Scheme 1. Structures of the chloroquine enantiomers (free bases) and of iron-protoporphyrin IX.

recorded on a Perkin-Elmer (Norwalk, CT) model 298 spectrophotometer. Mass spectral data were obtained from a Hewlett-Packard (San Jose, CA) GC-MS equipment (HP-5890A, HP-5970C, HP-59970). Elemental analyses were performed by Mag. Theiner, Institute of Physical Chemistry, University of Vienna. High resolution ms were performed by Prof. Nikiforov, Institute of Organic Chemistry, University of Vienna. Flash chromatography was carried out using silica gel 60. Specific rotations were measured on a Perkin-Elmer (Norwalk, CT) 241 Polarimeter.

(–)-(R)-1-Benzoyl-5-methyl-2-pyrrolidone (3a). A solution of 500 mg (5 mmol) of (R)-5-methyl-2-pyrrolidinone **(2a)**[6] in 10 ml of dry THF was placed into a bath of 50°C. Benzoyl chloride (0.9 ml, 7.5 mmol) and triethylamine (1.1 ml, 7.5 mmol) were added dropwise and stirring was continued overnight at 50°C. After cooling, the formed precipitate was filtered off with suction and washed with diethylether. The combined filtrates were evaporated under reduced pressure. Purification of the residue by column chromatography (diethylether) yielded 770 mg of **3a** as spontaneously crystallizing oil (76%), recrystallization from diethylether, mp 74–76°C. ^{1}H-nmr (300 MHz): δ (ppm) = 7.65–7.30 (5H, m, arom.), 4.58–4.42 (1H, m, 5-H), 2.72–2.16, 1.80–1.66 (3H, 1H, m, m, 3-H, 4-H), 1.40 (3H, d, J = 6.3 Hz, 5-CH_3); ^{13}C-nmr (75 MHz): δ (ppm) = 174.6, 170.7 (C = O), 135.1, 131.7, 128.7, 127.7 (arom.), 53.7 (5-C), 31.6, 25.8 (3-C, 4-C), 19.8 (CH_3); ir: 1,745, 1,660 cm^{-1} (ν_{imide}); ms (m/z) = 203 (M^+). Anal. Calcd. for $C_{12}H_{13}NO_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.79; H, 6.63; N, 7.00. $[\alpha]^{20}_D$: –177.1° (c = 1.013, EtOH).

(+)-(S)-1-Benzoyl-5-methyl-2-pyrrolidone (3b). Using (S)-5-methyl-2-pyrrolidinone **(2b),** this compound was prepared as described for **3a** with a yield of 83%. Anal. Found: C, 71.03; H, 6.20; N, 6.85. $[\alpha]^{20}_D$: +177.7° (c = 1.149, EtOH).

(–)-(R)-N^4-Benzoyl-4-aminopentanoic acid diethylamide (4a). To a suspension of 870 mg freshly sublimed $AlCl_3$ (6.5 mmol) in 35 ml of dry 1,2-dichloroethane 1.015 g of lactam **3a** (5 mmol) and 1.3 ml of diethylamine (12.5 mmol) were added at 0°C. After stirring at room temperature for 5 h, a mixture of ice and water was added, followed by 2N HCl. The aqueous phase was extracted with dichlo-



Scheme 2. Synthesis of (–)-(R)-CQ-diphosphate, starting from (S)-pyroglutamic acid.

romethane 4 times, the combined organic fractions were washed with saturated sodium bicarbonate solution, dried over Na_2SO_4, and evaporated. Purification of the residue by column chromatography (diethyl ether) yielded 870 mg **4a** as a colorless oil (63%). ^{1}H-nmr (300 MHz): δ (ppm) = 7.82 (2H, d, J = 6.9 Hz, arom.), 7.50–7.35 (3H, m, arom. H), 4.19–4.03 (1H, m, 4-H), 3.38–3.16 (4H, m, N-CH_2CH$_3$), 2.58–2.45, 2.44–2.31, 2.18–2.02, 1.96–1.78 (5H, each m, 2-H, 3-H, NH), 1.30 (3H, d, J = 6.3 Hz, 5-H), 1.1, 1.0 (3H, 3H, each t, J = 6.9 Hz, N-CH$_2$CH_3); ^{13}C-nmr (75 MHz): δ (ppm) = 172.2, 166.6 (C = O), 134.6, 130.8, 128.0, 126.8 (arom.), 46.5 (4-C), 41.8, 40.2 (N-CH$_2$CH$_3$), 30.5, 29.9 (3-C, 4-C), 21.1 (5-C), 13.9, 12.7 (N-CH$_2$$CH_3$); ir: 1,640 cm^{-1} ($\nu_{amide}$); ms (m/z) = 276 (M$^+$). Anal. Calcd. for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14. Found: C, 69.31; H, 8.77; N, 9.98. $[\alpha]^{20}_D$: –23.7° (c = 0.802, EtOH).

(+)-(S)-N^4-Benzoyl-4-aminopentanoic acid diethylamide (4b). Using the procedure described for **4a,** the diamide derived from **3b** was obtained as a colorless oil with 55% yield. High resolution ms: Calcd. for $C_{16}H_{24}N_2O_2$: 276,183; Found: 276,183. $[\alpha]^{20}_D$: + 25.5° (c = 0.945, EtOH).

(–)-(R)-N^4-Benzyl-N^1,N^1-diethyl-1,4-pentanediamine (5a). To a solution of 1.38 g of **4a** (5 mmol) in 50 ml of dry THF, 12.5 ml of a 1M solution of LAH in THF (12.5 mmol) was added slowly. The mixture was refluxed for 4 h. After cooling, water was added dropwise with vigorous stirring, the formed precipitate was filtered off with suction and washed with diethylether. The solvent was evaporated to give an oily residue, which was purified by column chromatography (ethyl acetate/triethylamine = 9/1) to give 840 mg **5a** as colorless oil (68%). ^{1}H-nmr (80 MHz): δ (ppm) = 7.30, 7.45–7.10 (s, m, 5H, arom.), 3.80 (dd, AB-system, 2H, J = 1.6 Hz, J = 13.0 Hz, benzyl.), 2.85–2.25 (m, 7H, 1-H, 4-H, N-CH_2 CH$_3$), 1.70 (br s, 1H, NH), 1.60–1.20 (m, 4H, 2-H, 3-H), 1.17–0.90 (d, t, t, 9H, CH$_3$), ^{13}C-nmr (75 MHz): δ (ppm) = 140.9, 128.1, 127.9, 126.5 (arom.), 53.2 (benzyl.), 52.3 (4-C), 51.2 (1-C), 46.8 (N-CH$_2$CH$_3$), 35.0 (3-C), 23.5 (2-C), 20.3 (5-C), 11.6 (CH$_2$$CH_3$); ir: 3,450 cm^{-1} ($\nu_{NH}$); ms (m/z) = 248 (M$^+$). Anal. Calcd. for $C_{16}H_{28}N_2$: C, 77.36; H, 11.36; N, 11.28. Found: C, 77.55; H, 11.40; N, 11.37. $[\alpha]^{20}_D$: –1.4° (c = 1.385, EtOH).

(+)-(S)-N^4-Benzyl-N^1,N^1-diethyl-1,4-pentanediamine (5b). Using the procedure described for **5a,** the diamine derived from **4b** was obtained as a colorless oil with 75% yield. Anal. Found: C, 77.13; H, 11.53; N, 11.07. $[\alpha]^{20}_D$: +1.2° (c = 0.242, EtOH).

(–)-(R)-CQ-diphosphate. A solution of 1.24 g (5 mmol) of **5a** in 80 ml methanol was treated with hydrogen in the presence of 200 mg of 10% palladium on carbon as catalyst for 10 h. The mixture was filtered through Celite and the solvent evaporated to give 640 mg of **(R)-4-Amino-1-(diethylamino)-pentane** as colorless oil (81%), which was directly converted to **(–)-(R)-CQ** according to the literature.[3,8] $[\alpha]^{20}_D$: –108.7° (c = 1.050, EtOH). The preparation of the **(–)-(R)-CQ-diphosphate** followed the published method.[9] Anal. Calcd. for $C_{18}H_{32}ClN_3O_8P_2$: C, 41.91; H, 6.25; N, 8.15. Found: C, 41.74; H, 6.06; N, 7.95. $[\alpha]^{22}_D$: –88.6° (c = 0.571, H_2O).

(+)-(S)-CQ-diphosphate. Using the procedure described above, **(S)-4-Amino-1-(diethylamino)-pentane** was prepared with a yield of 93% and gave **(+)-(S)-CQ** with $[\alpha]^{20}_D$: +107.5° (c = 1.169, EtOH). For **(+)-(S)-CQ-diphosphate:** Anal. Found: C, 41.85; H, 6.03; N, 7.97. $[\alpha]^{22}_D$: +88.6° (c = 0.502, H_2O).

Hemin. (ferriprotoporphyrin IX chloride) was purchased from Sigma (Bovine, Type I). Stock solutions of about 10 mg hemin in 25 ml 0.02 N aqueous NaOH were stored for up to 2 weeks at 0° to 4°C in plastic vessels and were protected from light. Buffers and other electrolytes were of analytical grade.

Chloroquine diphosphate solutions. Aqueous solutions (1.5 to 1.8) × 10^{-3} M of CQ were stored at 0° to 4°C in plastic vessels and in the dark.

Preparation of the complexes. Usually, an alkaline stock solution of FP [(7 to 8) × 10^{-4} M] was added to an aqueous solution of buffer and NaCl. The pH was adjusted to a required value, using concentrated HCl or NaOH. Finally, a required amount of a CQ stock solution was added to a given volume (usually 2 ml). Plastic vessels were used in order to minimize adsorption of the CQ or FP to the walls of the vessel. Solutions were protected from light. pH values were checked at the end of a measurement. In most cases, they remained constant within about 0.1 pH unit. In most experiments reported, no turbidity of the solutions was observed, unless indicated otherwise.

Light-absorption spectra were measured at room temperature on a Hewlett-Packard 8452A Diode Array Spectrophotometer. The reference solvent was water in most cases. There were no appreciable differences in absorption down to about 250 nm if the reference solvent contained sodium chloride and buffer at the concentrations indicated below.

Circular dichroism was measured on a Cary Model 60 recording spectropolarimeter equipped with a Model 6002 CD accessory. The instrument was calibrated daily with (+)-10-camphor sulfonic acid (Eastman Kodak, Rochester, NY; highest purity). Both the complexes or free drugs were measured in the same cell as the reference solvent (water). The optical path of the cells was chosen to give absorbance values not exceeding 1.2 in most experiments. The temperature in the cell compartment was 27.0 ± 1°C. Molar ellipticities given were calculated according to: $[\theta]_\lambda = 10\theta_\lambda/c \cdot d$, where θ_λ is the observed ellipticity in degrees at wavelength λ; c, the concentration in moles per liter of total FP or CQ; and d, the cell path length in decimeters. As reported in previous work,[1] aqueous solutions of 2 × 10^{-4} M FP did not show measurable ellipticity in the range of 300 to 650 nm at both pH 7.4 and 11.5, as would be expected.

RESULTS AND DISCUSSION

Light Absorption

CQ only. The light-absorption spectrum in the range of 200 to 400 nm of CQ alone at pH 7.3 is shown in Figure

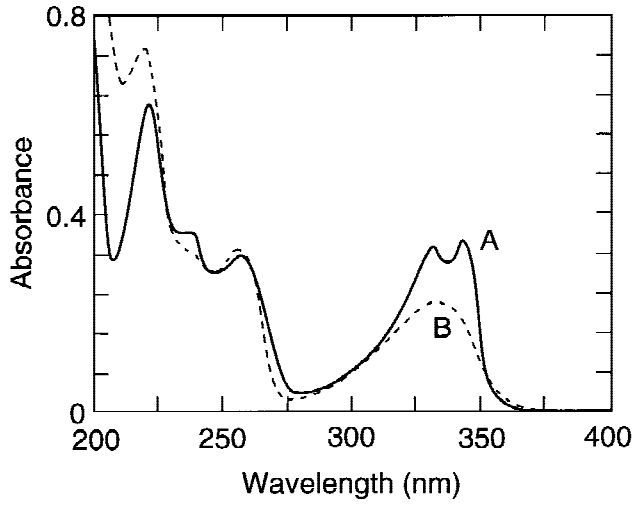


Fig. 1. Light-absorption spectra of CQ at two different pH-values. (+)-CQ, 1.0×10^{-4} M; NaCl, 0.1 M. Cell path length, 0.2 cm. Measured about 30 min after preparation of the solutions. A: pH 7.3, 0.01 M phosphate buffer was included. B: pH 11.3.

1(A). There are two close maxima of about equal absorption at 344 and 332 nm, respectively, in addition to peaks at 258 and 222 nm and a shoulder between them. Beyond about 365 nm, there is no measurable absorption. Under the conditions given, average ϵ-values of the near-UV maxima were $16.0 \pm 0.6 \times 10^3$ $M^{-1} \cdot cm^{-1}$ at 344 nm and $15.5 \pm 0.6 \times 10^3$ $M^{-1} \cdot cm^{-1}$ at 332 nm (average of 7 experiments with both enantiomers) as measured for (1 to 5) $\times 10^{-4}$ M solutions. A corresponding spectrum of CQ at pH 11.3 is shown in Figure 1(B). In this case, only one broad band is observed in the near-UV region with a maximum at 332 nm ($\epsilon \approx 11 \times 10^3$ $M^{-1} \cdot cm^{-1}$). The shorter wave-length maxima remain approximately at the same positions as at pH 7.3. The integrated band area between about 300 and 400 nm is smaller at pH 11.3. This may indicate specific aggregation of the CQ at alkaline pH. It has also been observed that CQ becomes insoluble in aqueous alkaline media at higher concentrations of the drug (see also reference 10).

Similar absorption spectra of CQ have been reported many years ago, as measured at pH 1.0, 8.1, and 12.0, respectively.[10]

FP-CQ complex. A typical light-absorption spectrum in the range of 250 to 650 nm of FP both in the absence and presence of CQ at pH 7.3 (molar ratio FP: CQ of 2:1) is shown in Figure 2. (Analogous absorption spectra measured at the same pH but at a molar ratio FP:CQ of 1:1 and in the absence of added 0.1 M NaCl have previously been reported[4]). While the near-UV absorption spectra of the FP-CQ system show two bands of about equal intensity with maxima at 390–392 and 344–346 nm, respectively, FP alone shows a single maximum at 364–366 nm with higher absorbance. In contrast, in the visible region the complex shows a pronounced maximum at about 600 nm, while FP alone shows a diffuse spectrum of significantly lower absorbance. All these spectral differences between the FP-CQ system and free FP confirm complex formation (see reference 4) between FP and the drug under the conditions given. Measurements at pH 11.3 are presented in Figure 3. In this case, the difference in absorbance between the FP and the FP-CQ system is much larger in the near-UV region, again with absorption peaks in the presence of CQ at 388–390 and 344–346 nm, respectively, and at 388 nm for free FP. The integrated band area in the Soret region is considerably smaller in the complex. In the visible region, an absorption maximum near 490 nm of free FP is absent in the complex and another maximum at 612 nm is shifted to 600 nm in the complex. The absorbance difference between free FP and FP-CQ at 600 nm is smaller than at pH 7.3. Therefore, under the present conditions, complex formation is conveniently measured at 390 nm at pH 11.3 and at either 364 or 600 nm at pH 7.3.

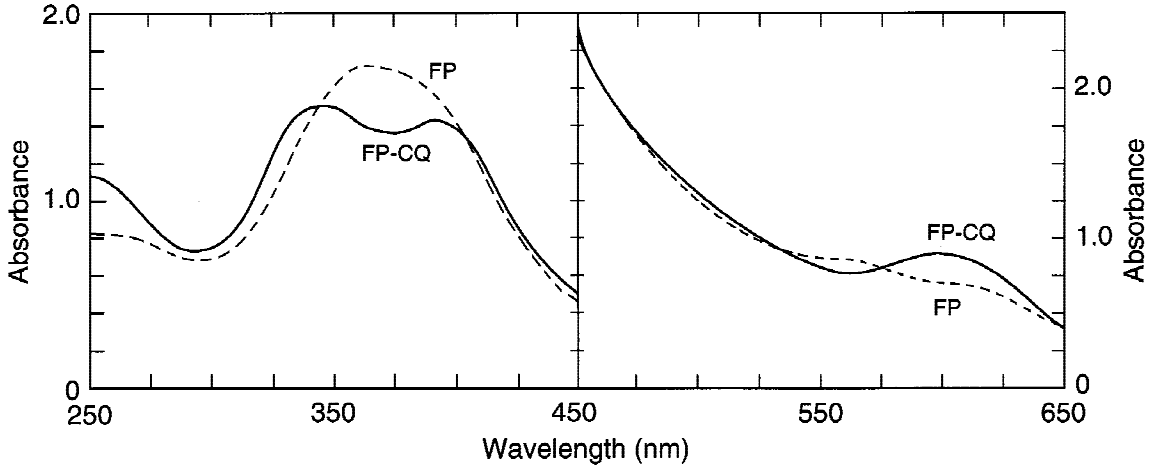


Fig. 2. Light-absorption spectra of FP in the absence and presence of CQ at pH 7.3. FP, 2.0×10^{-4} M; (+)-CQ, 1.0×10^{-4} M; NaCl, 0.1 M; phosphate buffer, 0.01 M. **Left:** Cell path length, 0.2 cm. **Right:** Cell path length, 1.0 cm. Measurements were started about 30 min after preparation of the solutions.

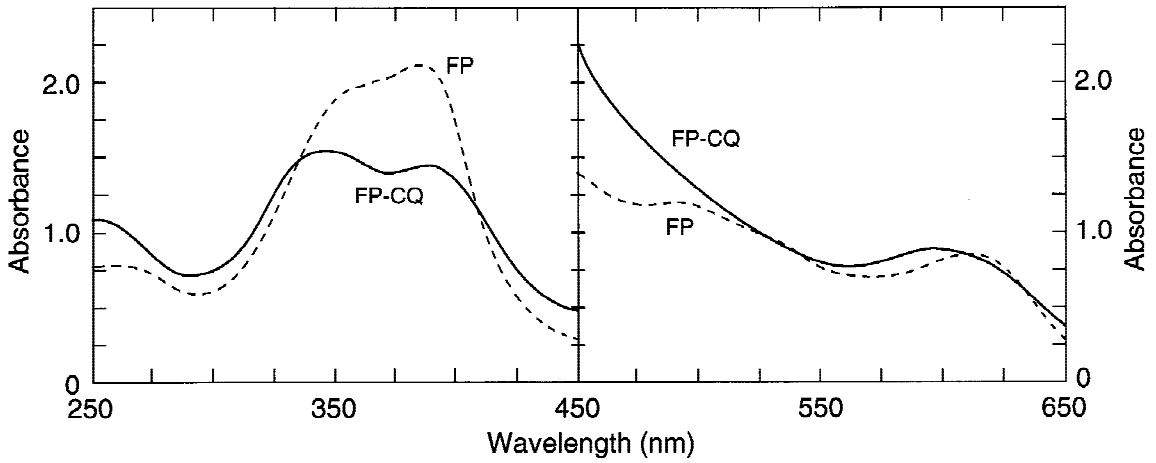


Fig. 3. Light-absorption spectra of FP in the absence and presence of CQ at pH 11.3. FP, 2.0×10^{-4} M; (+)-CQ, 1.0×10^{-4} M; NaCl, 0.1 M. **Left:** Cell path length, 0.2 cm. **Right:** Cell path length, 1.0 cm. Measurements were started about 30 min after preparation of the solutions.

Spectrophotometric titrations. Titrations were carried out at constant FP concentration at both pH 7.3–7.4 and 11.3 (Fig. 4). At pH 7.3–7.4, the absorbance at 600 nm is increased by addition of CQ (see Fig. 2) and reaches a constant value at a mole ratio FP:CQ of 2:1. At pH 11.3, the absorbance at 390 nm first decreases with increasing CQ concentration (see Fig. 3). Beyond a mole ratio FP:CQ of 2:1 the absorbance increases, probably due to some precipitation and associated turbidity caused by an excess of CQ (see above, solubility of CQ). Nevertheless, a predominant mole ratio of FP:CQ of 2:1 is apparent also at pH 11.3.

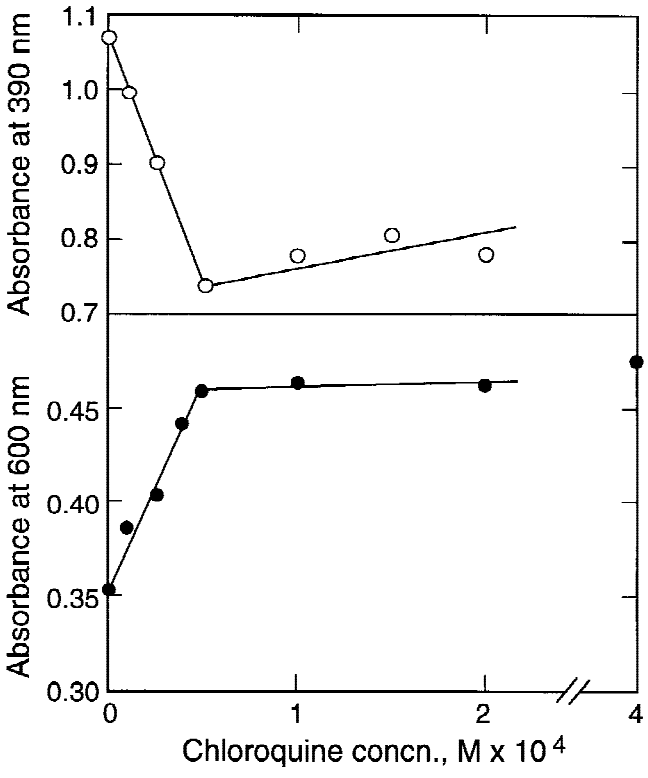


Fig. 4. Spectrophotometric titrations of FP with (+)-CQ at two different pH values. FP, 1.0×10^{-4} M; NaCl, 0.1 M. **Bottom:** pH 7.3–7.4; cell path length, 1.0 cm. **Top:** pH 11.3; cell path length, 0.2 cm. At pH 7.3–7.4, 0.01 M phosphate buffer was included; solutions were measured 20–30 min after preparation and at pH 11.3 after 10–15 min in most cases. Each point represents a separate experiment.

Spectrophotometric titrations were also carried out while keeping the total molar concentration of FP and *rac*-CQ constant and varying the mole fractions of the components.[11] Results obtained at pH values of 7.3, 11.3, and 12.0 also indicated a mole fraction of 0.66 of FP in the complex (not shown). Similar results have previously been reported by using equilibrium dialysis at neutral pH.[5] Thus, within the limitations of all titrations described[12] there appears to be sufficient evidence for a predominant mole ratio of 2:1 in the FP-CQ complex under the given conditions. It will be shown (see Fig. 6) that the same result is obtained at alkaline pH by measuring the ellipticity as a function of the FP mole fraction in the FP-CQ system.

Optical Activity

CQ only. $[\alpha]_D$-values for each of the enantiomers of CQ are given in Materials and Methods. CD measurements of both enantiomers were carried out at pH 7.3 in the range of 220–400 nm and are summarized in Table 1 for the main bands. As expected, the CD bands of the two enantiomers appear to be mirror images within the limits of error.

FP-CQ complex. As mentioned in Materials and Methods, aqueous alkaline solutions of FP do not exhibit optical activity. However, in the presence of sufficient concentrations of optically active drugs such as (–)-quinine,[1] (+)-quinidine,[1] or (–)-morphine,[13] large CD bands are observed in the Soret-band region of FP where the drugs do not show light absorption. In the present case, smaller CD bands were measured in the Soret region in the presence of optically active CQ at alkaline pH. The two enantiomeric forms of CQ exhibit approximate mirror-image CD bands around 410 nm (Fig. 5). However, each of the bands may

TABLE 1. Observed main CD-band extrema of (+)-(S)-CQ and (–)-(R)-CQ in aqueous solution, pH 7.3*

CQ enantiomer	Extremum (nm)	Molar ellipticity ($[\theta] \times 10^{-4}$ deg · cm^2 · dmol^{-1})[a]	Extremum (nm)	Molar ellipticity ($[\theta] \times 10^{-4}$ deg · cm^2 · dmol^{-1})[a]
(+)-(S)	264 ± 1	1.8 ± 0.2[b]	236 ± 1	1.50 ± 0.16[b]
(–)-(R)	263 ± 0.5	–1.78 ± 0.02[c]	234 ± 1	–1.43 ± 0.17[c]

*CQ, 2.0 to 5.0 × 10^{-4} M; NaCl, 0.1 M; phosphate buffer, 0.01 M. Measured in 0.1-cm cells 15–30 min after preparation of the solutions.
[a]Based on CQ.
[b]Three experiments.
[c]Two experiments.

be composite to some extent. A molar ratio of FP:CQ of 2:1 is used, since this appears to be the main composition of the complex (see above and the following CD titration). As shown in Table 2, apparent molar ellipticities of about ± 6 × 10^4 deg · cm^2 · dmol^{-1} (based on total FP) are measured at about 410 nm for the CD bands of opposite sign. Corresponding rotational strengths were about ± 0.5 Debye-Bohr magnetons.[a]

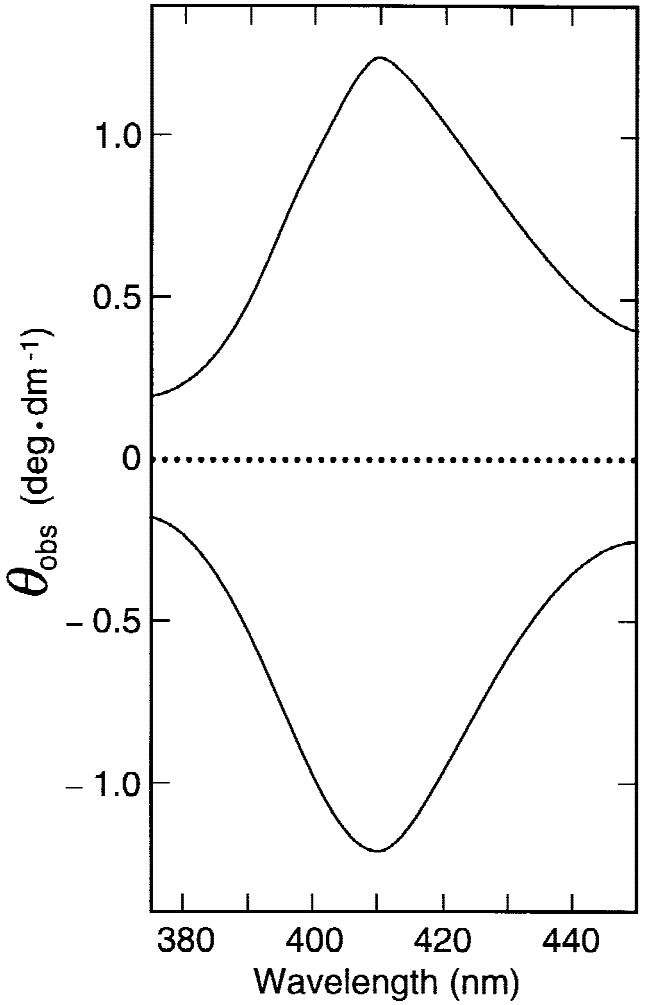


Fig. 5. CD spectra of FP in the presence of CQ enantiomers at pH 11.3. FP, 2.0 × 10^{-4} M; NaCl, 0.1 M. **Top:** (+)-CQ, 1.0 × 10^{-4} M. **Bottom:** (–)-CQ, 1.0 × 10^{-4} M. Measurements in 0.1-cm cells were started 20 to 30 min after preparation of the solutions. After a few hours, the ellipticities decreased significantly.

No additional bands were detected between 450 and 550 nm. In the UV region, smaller CD bands were observed around 325 to 330 nm [positive for FP-(–)-CQ and negative for FP-(+)-CQ], and also near 230 nm [negative for FP-(–)-CQ) and positive for FP-(+)-CQ]. At pH 12.0, and under otherwise identical conditions, a similar molar ellipticity extremum at 408 nm of –6.1 × 10^4 deg · cm^2 dmol^{-1} was obtained for FP-(–)-CQ as at pH 11.3. However, at pH 7.3, no significant ellipticity was measured near 400 nm.

The CD bands observed at 410 nm can conveniently be used for independent determination of the mole fraction of FP in the complex, since neither free FP nor optically active, free CQ will contribute to this optical activity. By analogy with titrations by light absorption (see above), ellipticity values were recorded at different mole fractions of FP, keeping the total molar concentrations of the complex components constant (Fig. 6). In the present case, (–)-CQ was used at pH 11.2 to 11.3, yielding a distinct extremum of the observed ellipticity at a mole fraction FP of 0.66, in agreement with the spectrophotometric data described above.

Ultracentrifugation

In previous measurements of the FP-CQ system (each component 4 × 10^{-4} M) in the analytical ultracentrifuge, specific aggregates were observed at both pH 7.4 and 12, with apparent s_{20}-values of 4 to 5 S.[4] These values are relatively small as compared to the sedimentation coefficients of the FP-quinine and FP-quinidine complexes[1] (see also reference[13]).

Origin of the Optical Activity and Structure of the FP-CQ Complex

Previously investigated analogous FP-drug systems showed absolute values of molar ellipticities in the Soret region, which, under certain conditions, were larger by a factor of about 5 in the case of FP-(–)-quinine,[1] by a factor of about 8 to 9 for FP-(–)-morphine,[13] and by about two orders of magnitude for FP-(+)-quinidine complexes.[1] The

[a]These values were estimated from the data of Figure 5 and the approximate relation for the rotational strength $R_k \approx 1.23 \times 10^{-42}\ [\theta_k^\circ]\ \Delta_k/\lambda_k^\circ$ (cgs) where $[\theta_k^\circ]$ is the molar ellipticity at a band extremum of a transition k, Δ_k is the band half-width at $[\theta_k^\circ]/e$, and λ_k°, the wavelength of the band extremum.[14]

TABLE 2. Observed main CD-band extrema of FP-(+)-CQ and FP-(–)-CQ in aqueous solution, pH 11.3*

CQ enantiomer	Extremum (nm)	Molar ellipticity ($[\theta] \times 10^{-4}$ $deg \cdot cm^2 \cdot dmol^{-1}$)[a]
(+)-(S)	409 ± 1	5.9 ± 0.3[b]
(–)-(R)	410	–6.3 ± 0.2[b]

*FP, 2.0×10^{-4} M; CQ, 1.0×10^{-4} M; NaCl, 0.1M. Measured in 0.1-cm cells, 20–30 min after preparation of the solutions.
[a]Based on FP.
[b]Two experiments.

kinetics of formation were also different for the latter complex. Corresponding rotational strengths were larger by a factor of about 25 in the latter case as compared to the present FP-CQ complex. For all the above-mentioned complexes, mole ratios of 1:1 were observed, as compared to the present ratio FP:CQ of 2:1. In the previously investigated systems, adjacent Soret CD bands of opposite sign and very large aggregates were observed, while in the present case, a band of either positive or negative sign and of a relatively small rotational strength was measured. Therefore, the concept of exciton-type interactions between FP molecules within chiral arrays of large aggregates of FP-drug complexes[1,13] cannot be applied to the present FP-CQ system. The absence of exciton-type interactions in the FP-CQ complexes (mole ratio FP:CQ of 2:1) may be due to the absence of such interactions between FP dimers within the complex aggregates. Local perturbations of the FP transitions by the chiral CQ molecules in the complex and/or coupling of electric transition dipole moments of FP with those of CQ within an aggregate may be considered as a possible source for the generation of optical activity, in addition to possible slight distortions from planarity in the bound FP.[15]

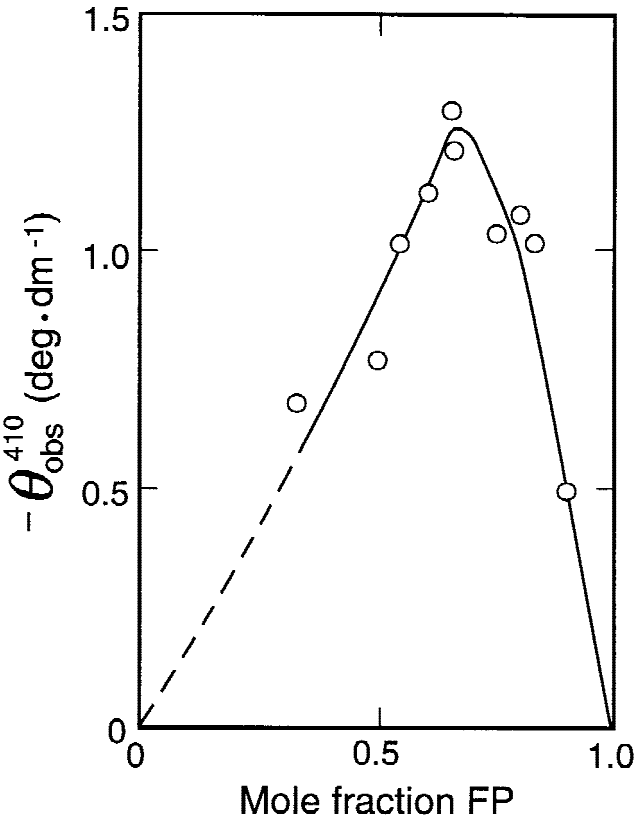


Fig. 6. Ellipticity as a function of varying FP and CQ concentrations, pH 11.2–11.3. Total constant concentrations of FP and (–)-CQ, 3.0×10^{-4} M; NaCl, 0.1 M. ○, Observed ellipticity at 410 nm. CD was measured in cells of 0.1-cm path length, 20 min after preparation of the solutions. Each point represents a separate experiment.

Regarding the stability of the FP-CQ aggregated complex, a variety of non-covalent interactions between the complex components should be considered, in particular hydrophobic and π-π electron interactions between the quinoline and FP ring systems. On the basis of NMR-data, a model of CQ stacking between μ-oxo-FP dimers within polymeric structures has been proposed,[16,17] which would be in agreement with the 2:1 mole ratio between FP and CQ. The protonated nitrogen atoms of CQ could interact electrostatically with the FP carboxylate groups at neutral pH, while this type of interaction should diminish at pH 11 to 12 where the nitrogen atoms should be, at least partially, non-protonated.[10] Possible steric differences in the aggregate complexes may, therefore, result at the different pH values. In the presence of chiral CQ, significant optical activity of the FP-CQ complex is observed in the present case only at higher pH.

LITERATURE CITED

1. Blauer, G. Optical properties of complexes of antimalarial drugs with ferriprotoporphyrin IX in aqueous medium. I. The system ferriprotoporphyrin IX-quinine. Arch. Biochem. Biophys. 251:306–314, 1986. II. The system ferriprotoporphyrin IX-quinidine. Arch. Biochem. Biophys. 251:315–322, 1986.
2. Blaschke, G., Kraft, H.-P., Schwanghart, A.-D. Chloroquin-Enantiomere durch chromatographische Racemattrennung und Synthese. Chem. Ber. 111:2732–2734, 1978.
3. Craig, J.C., Bhargava, H.N., Everhart, E.T., LaBelle, B., Ohnsorge, U., Webster, R.V. Absolute configuration of the enantiomers of 7-chloro-4-[[4-(diethylamino)-1-methylbutyl]amino]-quinoline (chloroquine). J. Org. Chem. 53:1167–1170, 1988.
4. Blauer, G., Ginsburg, H. Complexes of antimalarial drugs with ferriprotoporphyrin IX. Biochem. Int. 5:519–523, 1982.
5. Chou, A.C., Chevli R., Fitch, C.D. Ferriprotoporphyrin IX fulfills the criteria as the chloroquine receptor of malaria parasites. Biochemistry 19:1543–1549, 1980.
6. McIntosh, J.M., Acquaah, S.O. Azapropellanes 5. Synthesis of chiral (non-racemic) 2-methyl azapropellanes. Can. J. Chem. 66:1752–1756, 1988.
7. Bon, F., Bigg, D.C.H., Bertrand, G. Aluminium chloride promoted aminolysis of lactams. Synlett: 747–748, 1992.
8. Kenyon, R.L., Wiesner, J.A., Kwartler, C.E. Chloroquine manufacture. Ind. Eng. Chem. 41:654–662, 1949.
9. Drake, N.L., Creech, H.J., Draper, D., Garman, J.A., Haywood, S., Peck, R.M., Walton, E., Van Hook, J.O. Synthetic antimalarials. The preparation and properties of 7-chloro-4-(4-diethyl-amino-1-methylbutylamino)-quinoline (SN-7618). J. Am. Chem. Soc. 68:1214–1216, 1946.
10. Irvin, J.L., Irvin, E.M. Spectrophotometric and potentiometric evaluation of apparent acid dissociation exponents of various 4-aminoquinolines. J. Am. Chem. Soc. 69:1091–1099, 1947.
11. Brasted, R.C., Cooley, W.E. Physical methods in coordination chemistry. In: The Chemistry of Coordination Compounds. Bailar, J.C., Busch, D.H., eds. New York: Reinhold, 1956:563–624.

12. Sillén, L.G. Polynuclear complexes in solution. In: Coordination Chemistry, Vol. 1. Martell, A.E., ed. New York: van Nostrand Reinhold Co., 1971:491–541.
13. Blauer, G., Burla, R., Levy, S. Circular dichroism of ferriprotoporphyrin IX-morphine complexes in aqueous solution. Arch. Biochem. Biophys. 316:561–566, 1995.
14. Moscowitz, A. Theory and analysis of rotatory dispersion curves. In: Optical Rotatory Dispersion. Djerassi, C., ed. New York: McGraw-Hill, 1960:150–177.
15. Blauer, G., Sreerama, N., Woody, R.W. Optical activity of hemoproteins in the Soret region. Circular dichroism of the heme undecapeptide of cytochrome *c* in aqueous solution. Biochemistry 32:6674–6679, 1993.
16. Moreau, S., Perly B., Biguet, J. Interactions de la chloroquine avec la ferriprotoporphyrin IX. Biochimie 64:1015–1025, 1982.
17. Moreau, S., Perly, B., Chachaty, C., Deleuze, C. A nuclear magnetic resonance study of antimalarial drugs with porphyrins. Biochim. Biophys. Acta 840:107–116, 1985.