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^{1}H-NMR studies on the self-association of chloroquine in aqueous solution

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The concentration dependences of ^{1}H-NMR chemical shifts and spin-lattice relaxation rates were measured for chloroquine in aqueous solution. The weak self-association constant was evaluated according to a dimerization equilibrium with the formation of self-stacked adducts ($\overline{K}_d = 4.52 \pm 0.68$ l mol^{-1}). The motional correlation times were evaluated for the monomer and the dimer by measuring intramolecular dipolar cross-relaxation rates of aromatic vicinal protons ($\tau_{cm} = 0.06$ ns and $\tau_{cd} = 0.26$ ns). The geometry of the stacked dimer was elucidated by measuring intermolecular dipolar cross-relaxation rates and interpreted in terms of partial superposition of quinoline moieties.

1. Introduction

Weak complexes are important in molecular biology and in pharmacology for interpreting biomechanisms and modes of drug action. An important class of molecular complexes is that formed by molecules containing unsaturated π electrons and aromatic rings. It is, for example, generally considered that the stacking interaction of the π clouds of nucleotide bases is the primary stabilizing structural feature of the DNA double helix in water [1] and it is well known that nucleotide bases stack rather than form hydrogen-bonded pairs in aqueous solution [2].

NMR methods have been demonstrated to be important for the study of molecular complexation in solution, since self-stacking or charge-transfer complexation yields considerable changes in the ^{1}H- or ^{13}C-NMR spectra, from which the association constant and electronic structure of the stacked adduct can be calculated [3].

In the present contribution, a ^{1}H-NMR study on the self-association of chloroquine (7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline) (fig. 1) is reported. The crystal structure of this antimalarial drug is characterized by a zigzag packing similar to those of benzene and naphthalene [4] and the quinoline nucleus is perfectly planar such that self-stacking in aqueous solution is very likely to occur.

The relevance of investigating the self-association behavior of chloroquine stems from the suggestion that the mode of action of the drug is due to binding to hematin [5] and also from the report that a high affinity of chloroquine for hy-

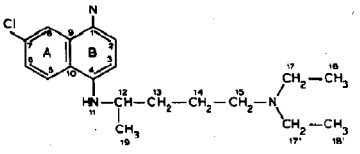

Fig. 1. Molecular formula of chloroquine.

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droxyferriprotoporphyrin IX has been measured [6]. In agreement with such findings, a ^{1}H-NMR study demonstrated close stacking of the porphyrin ring and the quinoline moiety of either chloroquine or quinine [7].

2. Materials and methods

Chloroquine was purchased from Sigma Chemical Co. and used without further purification. Solutions were made in ^2H_2O (99.9%, Merck) and were carefully deoxygenated by bubbling with nitrogen gas. The pH was adjusted with either ^{2}HCl or NaO^{2}H.

The NMR spectra were taken on a Varian VXR-200 NMR spectrometer in the pulsed-FT mode at probe temperatures of 295 ± 1 K; chemical shifts were referenced to the ^{1}H resonance of internal TSP-d_4. Non-selective spin-lattice relaxation rates were measured with the inversion recovery pulse sequence $(t-\pi-\tau-\pi/2)_n$. Single-selective and double-selective spin-lattice relaxation rates were measured with inversion recovery pulse sequences where the π pulse was given by the proton decoupler gated at the chosen frequencies for relatively long times (typically 20 ms). The $1/T_1$ $(= R_1)$ values were calculated, in the initial rate approximation [8,9], from the recovery curves of longitudinal magnetization components.

Two-dimensioned (2D) nuclear Overhauser effect (NOE) proton spectra were measured by using sequences described in ref. 10. The spectral width was 2000 Hz and the data set consisted of 1024 points in both dimensions for a total acquisition time of approx. 4 h.

3. Results and discussion

The 200 MHz ^{1}H-NMR parameters of chloroquine (50 mmol dm^{-3} in ^2H_2O) are reported in table 1. The chemical shifts of the ring protons are strongly concentration dependent, as shown in fig. 2. Ring A protons are shifted upfield on increasing the concentration, while ring B protons are shifted downfield. Moreover, ring B protons exhibit the larger 'absolute value' concentration-dependent shifts. The involvement of aromatic protons leads to the suggestion that self-association of chloroquine occurs via stacking, as also expected from the perfect planarity of the quinoline nucleus [4] and from the packing pattern in the solid state, that resembles those of benzene [11] and naphthalene [12].

Table 1

^{1}H-NMR parameters of chloroquine (5×10^{-2} mol dm^{-3}) in ^2H_2O (pH 7.0 *, $T = 295$ K)

Proton	δ (ppm)	J (Hz)
H_2	8.32	$J_{2,3} = 7.2$
H_5	8.22	$J_{5,6} = 9.0$
H_8	7.83	$J_{8,6} = 1.8$
H_6	7.63	
H_3	6.90	
H_{12}	4.17	$J_{12,19} = 6.7$
$H_{15,17,17'}$	3.20	$J_{17,18} = 7.2$
$H_{13,14}$	1.85	
H_{19}	1.44	
$H_{18,18'}$	1.27	

* pH-meter reading.

In the case of chloroquine, the observed shifts, either upfield or downfield, are somewhat smaller than those observed for purine and its derivatives

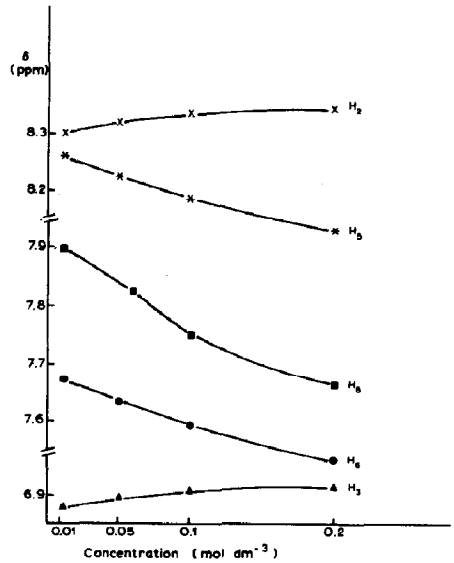

Fig. 2. Concentration dependence of ^{1}H-NMR chemical shifts of chloroquine in ^2H_2O at pH 7.0 and $T = 295$ K.

[3,13–15]; as a consequence, instead of considering the isodesmic or similar models for indefinite non-cooperative self-stacking [16,17], the concentration dependence was interpreted on the basis of a two-state model, assuming that the chloroquine molecules exist in either monomer or dimer forms. Accordingly, experimental data were analysed in terms of rapid exchange between the two states:

$$\delta_{obs} = x_m\delta_m + x_d\delta_d \quad (1)$$

where δ_m and δ_d denote the respective chemical shifts of the monomer and self-associated dimer forms with x_m and x_d being the corresponding fractions of chloroquine molecules in the two states ($x_m + x_d = 1$). The following equations were also considered:

$$K_d = \frac{[D]}{[M]^2}$$

$$x_m = \frac{[M]}{[M] + 2[D]} \quad (2)$$

$$x_d = \frac{2[D]}{[M] + 2[D]}$$

where [M] and [D] represent the molar concentrations of the monomer and dimer forms, respectively. The independent fitting of eqs 1 and 2 to each experimental data set yielded the self-association parameters given in table 2. The self-dimerization constants, with their standard deviations, calculated by measurements on the different ring protons, yield values rather close to each other, providing an average self-dimerization constant $\bar{K}_d = 4.52 \pm 0.68$ 1 mol^{-1}. Since the reference TSP-d_4 (trimethylsilylpropanoate) can affect the calculated K_d values through formation of ion-pairs with N_{16}, curve fitting was also performed on shift differences, e.g., that between H_2 and H_6, yielding K_d values consistent with those calculated from the data in table 2.

Table 2

Calculated parameters of self-dimerization for chloroquine in ^2H_2O at 295 K

Proton	K_d (1 mol^{-1})	δ_d (ppm)	δ_m (ppm)
H_2	4.48±0.70	8.40	8.30
H_5	4.27±0.59	8.06	8.26
H_8	4.41±0.66	7.51	7.90
H_6	4.03±0.60	7.46	7.67
H_3	5.19±0.83	6.98	6.87

The upfield shifts experienced by ring A protons, as in the case of ^{1}H-NMR of several other aromatic moieties, are commonly attributed to the predominance of ring-current-induced diamagnetic shielding effects from the opposing aromatic ring in the favored equiplanar geometry. The downfield shifts of ring B protons resemble those experienced by peripheral protons of naphthalene and anthracene in charge-transfer complexes with trinitrobenzene [18,19]. Since the peripheral positions were well within the shielding cone, the predominance of the opposing charge-transfer effect and the apparent tendency to transfer charge more effectively from the extremities of the donor molecules were suggested to account for the observed phenomenon. The same explanation may hold true in the case of chloroquine as well.

In order to determine the exact geometry of the self-stacked dimer, ^{1}H spin-lattice relaxation rates and nuclear Overhauser effects have been measured. The contour plot of the 2D magnetization transfer ^{1}H-NMR spectrum (shown in fig. 3 for 0.1 mol dm^{-3} chloroquine in water) clearly shows that ring B protons are connected in dipolar fashion to ring A protons in highly concentrated aqueous solutions. Such dipolar connectivities can potentially provide the most suitable approach for the evaluation of intra- and intermolecular proton-proton distances but (i) quantitation of cross-peaks in 2D NOE experiments may not be straightforward, (ii) 1D ^{1}H-{^{1}H} NOE measurements are usually affected by several systematic errors and (iii) ^{1}H-NMR spin-lattice relaxation rates cannot easily be interpreted. Several dipolar relaxation vectors can in fact be associated with any proton i, each relaxation vector having its own 'modulus' (determined by the proton-proton distance) and its own motional effective correlation time.

A method for isolating the cross-relaxation term between any two spins was suggested by Hall and Hill [20]; it relies on the possibility of selectively

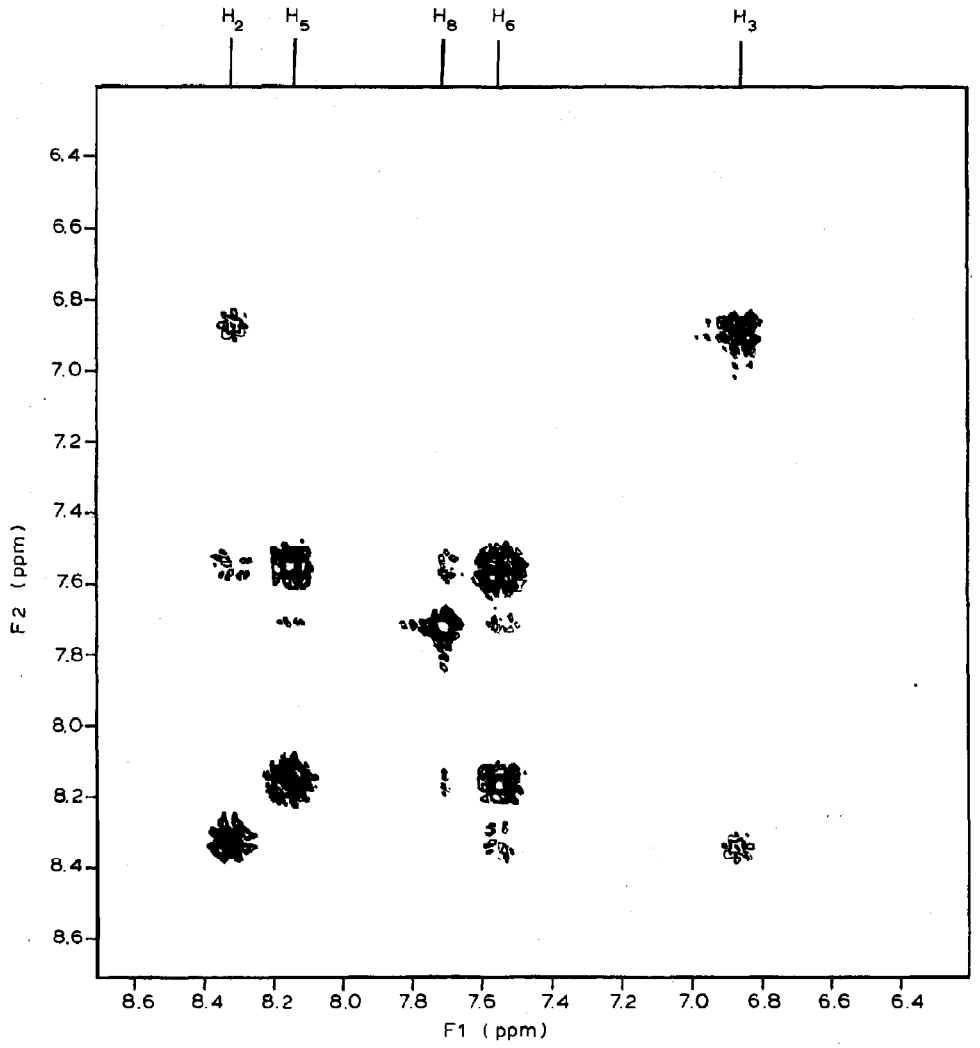

Fig. 3. 2D NOE ^{1}H-NMR spectrum of chloroquine (0.1 mol dm^{-3}) in ^2H_2O at pH 7.0 and $T = 295$ K. See text for experimental details.

exciting any two dipolar coupled spins while leaving all others unperturbed. It has been shown that the resulting 'double-selective' spin-lattice relaxation rates, as measured by the initial slopes of the recovery curves, can be suitably processed to yield the desired dipolar interaction term [9,20–22].

The concentration dependences of non-selective, single-selective and double-selective relaxation rates were measured for some aromatic protons of chloroquine, as exemplified in fig. 4 for H_2. All three relaxation rates are linearly dependent upon the molar fraction of the monomer (as calculated with the previously obtained $\bar{K}_d$), thus supporting the two-state model for self-association of chloroquine. The following equation can in fact be considered to account for the data:

$$R_{obs} = x_m R_m + x_d R_d = R_d + x_m (R_m - R_d) \quad (3)$$

where R denotes any relaxation rate. If indefinite self-association occurred one would expect a different concentration dependence. However, the slopes of the three straight lines are quite different

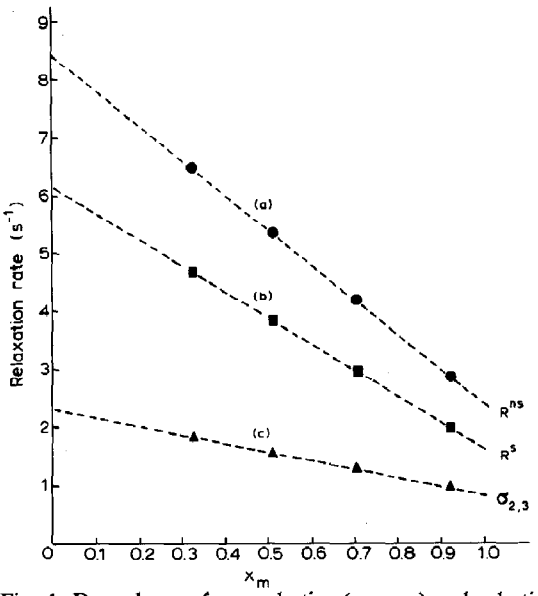

Fig. 4. Dependence of non-selective (curve a) and selective (curve b) spin-lattice relaxation rates of the H_2 proton as well as of the cross-relaxation rate of the H_2-H_3 spin-pair of chloroquine in ^2H_2O upon the molar fraction of the monomer at pH 7.0 and $T = 295$ K.

from each other, yielding evidence of some intermolecular dipolar interaction contributing to the relaxation mechanism. It is in fact expected that an intermolecular dipole-dipole interaction provides a certain contribution to non-selective and selective relaxation rates of aromatic protons within the dimer, as demonstrated by dipolar connectivities in 2D NOE spectra. As a consequence, consideration of R^{ns} or R^s yields different $R_m - R_d$ values in eq. 3, thus determining different slopes of the concentration dependences of either R^{ns} or R^s. This is not the case for $\sigma_{2,3}$ (as calculated from the difference between the double-selective and the selective relaxation rates) that is merely the cross-relaxation rate exclusively determined by the intramolecular dipole-dipole interaction between the two vicinal protons. Curve c (fig. 4) can therefore be fitted to the following equation:

$$\sigma_{obs} = \sigma_m x_m + \sigma_d x_d \qquad (4)$$

where σ_m and σ_d represent the cross-relaxation rates in the monomer and dimer, respectively.

Table 3

Nonselective (R^{ns}), single-selective (R^s) and double-selective ($R_i^{i,j}$) spin-lattice relaxation rates of aromatic protons of chloroquine (2×10^{-1} mol dm^{-3}) in ^2H_2O (pH 7.0, $T = 295$ K)

Proton	R^{ns} (s^{-1})	R^s (s^{-1})	$R_i^{i,j}$ (s^{-1})
H_2	0.78		
H_5	1.09		
H_8	0.20	0.19	
H_6	0.57	0.39	$R_6^{5,6} = 0.54$ $R_6^{2,6} = 0.42$
H_3	1.29	1.01	$R_3^{2,3} = 1.25$

Extrapolation of curve c to $x_m = 0$ and $x_m = 1$ therefore provides a means of calculating σ_d and σ_m, respectively. $\sigma_m = 0.08$ s^{-1} and $\sigma_d = 0.23$ s^{-1} were obtained for the H_2-H_3 as well as for the H_5-H_6 relaxation vectors. Since σ_m and σ_d refer to protons at fixed distances ($r_{2,3} = r_{5,6} = 2.43$ Å) [4,23], they only differ in the value of the reorientational correlation time. By considering the explicit form of σ_{ij} in terms of dipolar spectral densities for protons at fixed distances [24], the motional correlation times were evaluated, yielding $\tau_c = 0.063$ ns for the monomer and $\tau_c = 0.260$ ns for the dimer at room temperature. Once mo-

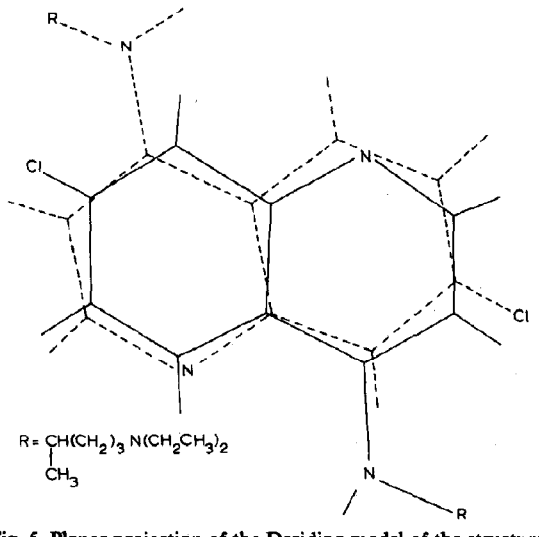

Fig. 5. Planar projection of the Dreiding model of the structure of the self-associated dimer of chloroquine in ^2H_2O at pH 7.0 at $T = 295$ K.

tional parameters have been independently obtained, the intermolecular dipolar interaction energy can be determined and used to obtain the geometry of the stacked dimer. Intermolecular cross-relaxation rates could in fact be obtained by measuring double-selective and single-selective spin-lattice relaxation rates of the protons involved. It turned out that only H_2 and H_6 constitute an intermolecular dipolar connected spin-pair (table 3), suggesting that ring B of one molecule faces ring A of the other molecule in the dimer. The geometry of the dimer can be specified by calculating the distance between H_2 and H_6. However, it should be borne in mind that H_2 and H_6 are not necessarily constrained at a fixed distance and, hence, that the internuclear distance is now averaged over all the eventually possible geometric assemblies [25]. Although this limitation of the method cannot be ignored, consideration of the motional correlation time of the dimer yields a value of $r_{2,6}$ ($r_{2,6} = 3.6$ Å) which is rather consistent with the geometry of other stacked adducts, e.g., those formed by purine nucleobases [3]. Since it is commonly accepted that interacting aromatic rings are found at a distance of 3.2 Å from each other, the measured value of $r_{2,6}$ suggests that the two quinoline rings are not exactly superimposed, but rather, that the two moieties are dephased, most probably due to steric hindrance by the diethylaminomethylbutylamino side chain. It should be mentioned that a different symmetry of the rotational diffusion tensor in the monomer and dimer can be expected to affect the evaluation of the correlation time based on isotropic reorientational behavior. This would imply that the calculated correlation times are 'effective' correlation times that are not simply related to the rotational diffusion constants. From this point of view, consideration of the same correlation time for the H_2-H_3 and H_2-H_6 relaxation vectors in the dimer can be taken as an approximation that, is, however, unlikely to affect severely the evaluated H_2-H_6 distance due to the r^{-6} dependence of the cross-relaxation rate. The NMR findings suggest a structure of the dimer similar to that shown in fig. 5 as a projection of the Dreiding model; the fact that self-association of chloroquine into stacked dimers occurs in the way shown by ^{1}H-NMR findings leaves the side chains free to extend in opposite directions.

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