unreported ^{13}CO chemical shift appears further downfield than in mammalian hemoglobins [17]. Thus, until specific values are published, what can be said is that elephant myoglobin conforms to the correlation of Fig. 2, in general terms. What can be concluded is that the position of elephant myoglobin–CO in the correlation indicates that the distal residue––CO interaction in this protein is enhanced over normal mammalian hemoglobins and myoglobins. That is, there seem to be 'better' distal ligands than the ubiquitous histidine E-7. A further conclusion is that ^{13}CO chemical shifts or infrared frequencies of CO-protein may be a quick, useful analytical technique for characterizing the distal amino acid in unsequenced b-type heme proteins.

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Q7

Chloroquine Interaction with Ferric Uroporphyrin in Solution

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It is estimated that, in 1979, 80% of the world's population was at risk to *Plasmodium* infection (malaria), primarily in the developing nations [1]. Chemotherapeutic agents useful in treatment include the quinine based drugs and the quinoline derivatives such as chloroquine [2, 3], although their molecular basis of action is not presently well understood. In patients undergoing malaria chemotherapy the drug is found in association with hemozoin pigments in the erythrocyte. Hemozoin is an aggregate of precipitated heme and denatured hemoglobin and this finding has stimulated our interest in the possibility that the antimalarial efficacy originated from association with heme complexes. Fitch *et al.* [4] have, in fact, suggested that erythrocyte localized protohemin IX is the putative receptor of chloroquine.

In this report we present some of our initial results concerning the interaction of chloroquine with iron porphyrins in solution. Urohemin was chosen due to its high solubility in aqueous solutions and because recent raman and nuclear magnetic resonance (NMR) work has allowed us to thoroughly characterize its solution dynamics [5].

Experimental

Urohemin was purchased from Porphyrin Products, Logan, Utah and was further purified by column chromatography. Chloroquine (Sigma) was used without further purification. Titrations of urohemin with the drug were carried out at pH 6 (unbuffered) employing a Cary 219 ultraviolet–visible spectrometer. pH was monitored throughout the experiment.

Results and Discussion

The data of Figs. 1 and 2 reveal that chloroquine does indeed associate with urohemin in solution. Changes in the optical spectrum (Fig. 1) occur, which may be used in an attempt to quantitate equilibrium behavior (Fig. 2).

The result of adding chloroquine to a solution of urohemin monomer (Fig. 1) is a spectrum in which the Soret intensity is lost, characteristic of the dimer (and higher order aggregates). However, unlike the

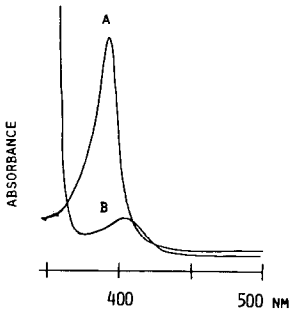

Fig. 1. Ultraviolet–visible spectra in the Soret region of: A, 5.0×10^{-6} *M* urohemin, pH 6.0, in H_2O. This is monomeric urohemin. B, 5.0×10^{-6} *M* urohemin and 4.89×10^{-3} *M* chloroquine, also in H_2O, pH 6.0. The decreased Soret intensity indicates association of chloroquine with urohemin and is characteristic of the decreased intensity observed for urohemin dimer.

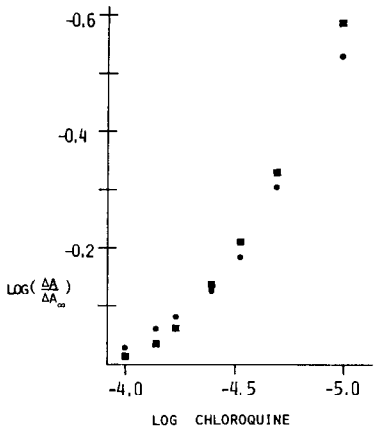

Fig. 2. Analysis of uv–visible data for equilibrium titrations of urohemin with aliquots of chloroquine solution. A standard equation was used from references [6, 7]. Abbreviations: ΔA is the absorbance difference at a wavelength maximum between urohemin alone and urohemin with various aliquots of chloroquine. ΔA_∞ is the absorbance difference between the complex (limiting absorbance) and urohemin with aliquots of chloroquine. This analysis is for a single equilibrium process and nonlinearity in this plot indicates the presence of multiple equilibria. Concentrations: urohemin (initial) 5.0×10^{-6} *M*; chloroquine (maximum) 4.89×10^{-3} *M*.

dimer spectrum chloroquine binding induces a shift in the Soret maximum to longer wavelengths.

Attempts to characterize the chloroquine–urohemin association by standard uv–visible methods [6] revealed that this system cannot be characterized by a single equilibrium process. This is shown by the deviation from linearity of the data in Fig. 2 [6, 7]. This data is the result of successive additions of chloroquine to a solution of monomer urohemin. This process produced standard appearing difference absorption spectra with two isosbestic points between 350 and 500 nm. The fact that linearity in Fig. 2 is not observed suggests that multiple equilibria are present in this system.

Further work on malaria drug interactions with free hemins and heme proteins is in progress in our laboratories.

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Q8

Evidence for Sulphur Ligation in Ferricytochrome *c* at Alkaline pH

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The inactivation of ferricytochrome *c* at alkaline pH proceeds with a pK of 9.3 and it has been shown that the process involves a slow conformational change coupled to a real ionization with a pK of 11 [1]. Important indicators of the alkaline isomerization are the disappearance of the iron–sulfur charge transfer band at 695 nm [2], and the disappearance and formation of signals monitored by NMR [3] and EPR [4]. The prevalent view has been that Met-80, the sixth ligand, is replaced by another strong field ligand [3, 4]. However, the results described below strongly suggest that Met-80 remains ligated to the haem iron.

The ^1H NMR signals (270 MHz) arising from the haem methyl groups of the first alkaline form [3] are found to be composed of overlapping resonances from two slowly exchanging forms of ferricytochrome *c*. Either form can be made dominant over the other by raising the temperature to 330 K or by addition of 0.3 *M* perchlorate at pH 10. In the upfield region of the spectrum two very broad resonances between −8 and 10 ppm are assigned to the Met-80 methyl group in its two environments.