Identification of a Chloroquine Importer in *Plasmodium falciparum*

DIFFERENCES IN IMPORT KINETICS ARE GENETICALLY LINKED WITH THE CHLOROQUINE-RESISTANT PHENOTYPE

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We demonstrate that uptake of the antimalarial drug chloroquine is temperature-dependent, saturable, and inhibitable in *Plasmodium falciparum*. These features are indicative of carrier-mediated transport and suggest that a *P. falciparum*-encoded protein facilitates chloroquine import. Although both chloroquine-resistant and susceptible parasite isolates exhibit facilitated chloroquine uptake, the kinetics differ. Chloroquine-resistant parasite isolates consistently have an import mechanism with a lower transport activity and a reduced affinity for chloroquine. These differences in uptake kinetics are linked with chloroquine resistance in a genetic cross. These data suggest that changes in chloroquine import kinetics constitute a minimal and necessary event in the generation of the resistant phenotype. Competitive inhibition of chloroquine uptake by amiloride derivatives further suggests that chloroquine import is mediated by a plasmodial Na⁺/H⁺ exchanger.

The emergence and spread of chloroquine-resistant *Plasmodium falciparum* strains has turned hopes of radical malaria control into despair. When the affordable antimalarial drug chloroquine lost its efficacy, malaria resurged in many developing nations. Chloroquine acts against the intraerythrocytic *P. falciparum* stages that are responsible for the clinical manifestation of the disease. These stages feed on the erythrocyte’s hemoglobin, which is digested in acidic vacuoles (lysosomes). The toxic by-product heme is polymerized into non-toxic hemozoin.

*In vitro* experiments demonstrated that chloroquine at micromolar concentrations inhibits heme polymerization in extracts prepared from both chloroquine-sensitive (CQS) and chloroquine-resistant (CQR) parasite isolates (1, 2). In *vivo*, however, inhibitive levels of chloroquine are only achieved in the vacuoles of CQS parasite isolates, whereas CQR parasite isolates accumulate significantly less chloroquine (3–5). The reduced accumulation of chloroquine is thought to provide the basis of resistance, suggesting that CQR parasite isolates lower their lysosomal chloroquine concentration below that necessary to inhibit heme polymerization.

Two alternative models have been formulated to attempt to explain the differences in chloroquine accumulation. The first model is based on the observations that the Ca²⁺ channel blocker verapamil reverses chloroquine resistance by preventing chloroquine efflux from resistant cells (6, 7). This is phenotypically reminiscent of multi-drug resistance (mdr) in cancer cells, in which an overexpressed P-glycoprotein functions as an ATP-dependent pump, expelling a wide range of anticancer drugs (8, 9). It has been suggested that CQR parasites have acquired a chloroquine efflux mechanism by mutating or amplifying mdr-like genes (10, 11, 12). However, no linkage between the chloroquine-resistant phenotype and known *P. falciparum* mdr genes was found in a genetic cross (13).

The second model proposes that CQR parasite isolates have a weakened vacuolar proton pump (14). Chloroquine, which is acidotropic due to its chemical properties as a diprotic weak base, would not accumulate as efficiently in the vacuoles of resistant parasite isolates. This so-called weak base hypothesis is contradicted by the absence of detectable pH differences in the vacuoles of CQR and CQS parasite isolates (15, 16).

Both of these models rely on the assumption that chloroquine only enters the parasite by simple diffusion and do not consider the possibility of a chloroquine import mechanism. While several studies have speculated on the existence of such a chloroquine concentrating mechanism (16–19), conclusive experimental evidence in support of this proposition has yet to be presented. Investigating the mode of chloroquine uptake is of fundamental importance in understanding the mechanism of chloroquine resistance. If a parasite-encoded transporter mediates chloroquine uptake, then loss or reduction of this function would account for differences in chloroquine accumulation.

We have examined the mechanism of chloroquine uptake by *P. falciparum*-infected erythrocytes and found that this process is carrier-mediated. Furthermore, changes in chloroquine uptake kinetics segregate with the chloroquine-resistant phenotype in a genetic cross. Competitive inhibition studies with amiloride derivatives suggest that chloroquine import is mediated by a *P. falciparum* Na⁺/H⁺ exchanger.

**EXPERIMENTAL PROCEDURES**

**Materials**—[³H]Chloroquine diphosphate (18.8 Ci/mmol) and NCS-II tissue solubilizer were supplied by Amersham International and [methoxy-³H]Hinulin (305 mCi/g) by DuPont NEN. Chloroquine, 5-[N,N-dimethylamino]laurid and amiloride were purchased from Sigma. EIPA (5-[N-ethyl-N-isopropyl]amilorid) was a gift from Dr. H. J. Lang, Hoechst AG.

*P. falciparum* Cultures—The *P. falciparum* clones HB3, Dd2, and the progeny from the HB3xDd2 cross were cultured as described (20) and synchronized using the sorbitol method (21). The hematocrit and parasitemia were accurately determined using a Neubauer counting chamber and microscopic examination of Giemsa-stained thin blood smears, respectively. In all experiments, parasite cultures at a 5% hematocrit containing 4% trophozoites were used.

[³H]Chloroquine Uptake Assay—The kinetic studies on chloroquine uptake by *P. falciparum* were conducted using a modification of a
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previously described transport assay (22). Synchronized parasite cultures containing 4% trophozoites were collected and washed twice in reaction buffer (25 mM Na-HEPES, 122.5 mM NaCl, 5.4 mM KCl, 0.8 mM MgCl₂, 1.2 mM CaCl₂, 1 mM NaH₂PO₄, 5.5 mM glucose, pH 7.3). Cells were resuspended in reaction buffer at a concentration of 2.7 × 10⁸ erythrocytes/ml and aliquoted into 200-μl samples. [³H]Chloroquine was added at the appropriate concentrations and the samples were incubated at 37°C. After 5 min duplicate 75-μl aliquots were removed from each sample and diluted with an equal volume of ice-cold reaction buffer containing an 100-fold excess of nonradioactive chloroquine. These aliquots were immediately spun through a layer of melting point bath oil (15,000 g, 30 s) to separate the cells from the aqueous medium, which contained the unincorporated [³H]chloroquine. The aqueous phase was removed from each sample and its radioactivity was determined to obtain an accurate measurement of the [³H]chloroquine concentration in each reaction. The cell pellets were recovered by cutting the reaction tubes through the melting point bath oil layer (16). The tips of the tubes containing the cell pellets were placed in a larger 1.5-ml Eppendorf tube and incubated with 50 μl of ethanol and 25 μl of toluene solubilizer overnight at 55°C. The lysates were decolorized by the addition of 25 μl of 30% H₂O₂ and acidified by the addition of 25 μl of 1 N HCl. The lysates were transferred to scintillation vials and the radioactivity was measured using a liquid scintillation counter (TRICARB 2100 TR, Packard).

Each experiment was repeated on ice (4°C) to determine the extent of the nonspecific interaction with [³H]chloroquine. Typically this value was 0–10% of that measured at 37°C. This value was subtracted from that obtained at 37°C. The intercellular space of the pellets was determined using [methoxy-³H]inulin, as described (23). Chloroquine uptake was calculated as fentomoles of [³H]chloroquine incorporation per 1 × 10⁷ trophozoites/min (fmol/1 × 10⁷ parasites/min), using the formulas developed for the analysis of transport kinetics (22).

Briefly, [S]o = concentration of chloroquine in the reaction medium (nm) = (cpm/mg) of chloroquine in the aqueous medium/μl (specific activity of [³H]chloroquine expressed as cpm/mg). [S]i = amount of chloroquine in the cell pellet expressed in fmol/2 × 10⁷ erythrocytes = (total soluble radioactivity associated with the pellet, expressed as cpm/2 × 10⁷ erythrocytes)/specific activity of [³H]chloroquine, expressed as cpm/fmol). [V]i = intracellular space of the pellet (μl/2 × 10⁷ erythrocytes). [S]i = chloroquine which is outside cells in pellet (fmol/2 × 10⁷ erythrocytes) = [V]i × [S]o (fmol/μl). Sₗ = chloroquine inside cells in the pellet (fmol/2 × 10⁷ erythrocytes). Sₗ = Sₗ - Sₗ. The Sₗ values were normalized to 1 × 10⁷ parasites based on the hematocrit and the parasitemia. Apparent Kₘ and Jₘ values were calculated using computerized least squares fit methods (Sigma Plot, Jandel Corp.).

**Time Course of [³H]Chloroquine Incorporation—**Dd2 and HB3 cell suspensions containing 2.7 × 10⁸ erythrocytes per milliliter with a parasitemia of 4% trophozoites were prepared as described above and incubated at 37°C in reaction buffer containing 50 nM [³H]chloroquine. Duplicate aliquots were withdrawn at the time points indicated and processed as described above.

**Temperature Dependence of Chloroquine Uptake—**HB3 and Dd2 cell suspensions were prepared as described above, and resuspended in reaction buffer containing 50 nM [³H]chloroquine. Aliquots were incubated at different temperatures. The pH of the reaction buffer at different temperatures was maintained at 7.3 using 1 N NaOH. From each sample duplicate aliquots were withdrawn after 5 min and analyzed as described above. Uninfected erythrocytes were analyzed accordingly. [³H]Chloroquine incorporation was calculated as fentomoles per 2 × 10⁸ erythrocytes/min to compare infected with uninfected erythrocytes.

**Inhibition of [³H]Chloroquine Uptake by Nonradioactive Chloroquine and EIPA—**HB3 and Dd2 cell suspensions were prepared as described above. Reaction tubes were preloaded with 50 nM [³H]chloroquine and the appropriate concentrations of nonradioactive chloroquine or EIPA, respectively. Reactions were started by the addition of the cell suspension. Duplicate aliquots were removed from each sample after an incubation of 5 min at 37°C and analyzed as described above. Uninfected erythrocytes were analyzed accordingly. For competitive inhibition studies, reaction tubes were preloaded with the appropriate concentrations of [³H]chloroquine and EIPA before the cell suspensions were added.

**Chloroquine Binding Assay—**HB3 and Dd2 cell suspensions (see above) were incubated in reaction buffer containing 50 nM [³H]chloroquine and 25 nM to 50 μM nonradioactive chloroquine in a final volume of 200 μl at 4°C. After 30 min duplicate aliquots were removed from each sample and processed as described above. The radioactivity in each sample was determined and corrected for nonspecific binding of chloroquine, which was obtained in the presence of 50 μM nonradioactive chloroquine. These values were divided by the specific activity of chloroquine in each reaction (cpm/fmol). The resulting values correspond to the amount of specifically bound chloroquine, which was expressed as fentomoles/1 × 10⁷ parasites.

**RESULTS**

**Chloroquine Uptake Is Temperature-dependent and Saturable—**The kinetics of chloroquine uptake was investigated to determine if chloroquine enters *P. falciparum* by carrier-mediated transport or simple diffusion. Both processes are readily distinguishable, as, in contrast to simple diffusion, carrier-mediated transport would be temperature-dependent and saturable.

Two *P. falciparum* clones were investigated: Dd2, a CQR parasite from Indochina (IC₅₀ = 124 nM) (13); and HB3, a fully chloroquine susceptible clone from Honduras (IC₅₀ = 15 nM) (13). Uninfected erythrocytes were examined in parallel. All kinetic measurements were conducted with synchronized parasite cultures, containing trophozoites at a parasitemia of 4%. Chloroquine uptake was monitored using a [³H]chloroquine concentration of 50 nM, which inhibits growth of HB3 but not Dd2.

Under these conditions, the time course of chloroquine incorporation differs profoundly between the two parasite clones investigated (Fig. 1). In the CQS parasite clone HB3, the amount of internalized chloroquine increased with time until reaching saturation after 30 min. By contrast, the CQR parasite clone Dd2 indicated a complex biphasic relationship between [³H]chloroquine incorporation and time. After an initial uptake phase of about 15 min, a temporal decline in the internalized chloroquine was observed, consistent with the previous described efflux mechanism (7, 13). To obtain reliable and accurate kinetic data on chloroquine uptake, all the following studies were conducted in the initial linear phase, i.e. reactions were terminated after 5 min.

We then determined whether or not chloroquine uptake is temperature-dependent. To ensure an equal number of parasites in each experiment, synchronized cultures of both HB3
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and Dd2 were aliquoted and the uptake of [3H]chloroquine was monitored in both parasite clones at different temperatures. Chloroquine uptake revealed a profound temperature dependence in HB3, with an optimum at 40 °C (Fig. 2). A similar temperature dependence was observed for Dd2, although less chloroquine was taken up (Fig. 2). These data provide the first experimental evidence for a transport mechanism responsible for chloroquine uptake in P. falciparum.

To confirm this conclusion, it was tested if the chloroquine uptake is saturable. Aliquots of HB3 and Dd2 cell suspensions were mixed with different concentrations of nonradioactive chloroquine and 10 nM [3H]chloroquine to achieve a final chloroquine concentration of 10 nM to 500 μM. It was found that the chloroquine uptake is saturable, as indicated by the reduction of internalized [3H]chloroquine in the presence of an excess of nonradioactive chloroquine (Fig. 3).

These data demonstrate that chloroquine uptake by P. falciparum-infected erythrocytes is temperature-dependent and saturable. Both properties are indicative of carrier-mediated transport and suggest the existence of a specific parasite-encoded protein that facilitates chloroquine uptake. By contrast, no evidence for a chloroquine import mechanism was found in uninfected erythrocytes, confirming previous reports (24, 25). Chloroquine uptake by erythrocytes is temperature-insensitive and unsaturable, consistent with a diffusion-controlled process (Figs. 2 and 3).

Kinetic Properties of the Importer Correlate with Chloroquine Resistance—Although both HB3 and Dd2 seem to possess a chloroquine importer, quantitative differences in the transport capacity are apparent from the studies described above. This would imply that the kinetic properties of the transporter differ between the CQR and CQS parasite clones investigated.

To test this hypothesis, the initial velocity of chloroquine uptake was measured over a range of chloroquine concentrations. Aliquots of an HB3 or Dd2 cell suspension were incubated with increasing concentrations of [3H]chloroquine for 5 min at 37 °C. The amount of parasite-associated [3H]chloroquine was determined and normalized to 1 x 10^6 parasites, as described under “Experimental Procedures.” The kinetics of initial chloroquine uptake can be described by the Michaelis-Menten equation in both HB3 and Dd2 (Fig. 4). Yet the kinetic parameters are unique to each of the two parasite clones (Fig. 4a). The maximal apparent transport rates V_max values observed are due to a change in the chloroquine transporter or its concentration, we measured the number of chloroquine-binding sites in HB3 and Dd2 using a competitive equilibrium binding assay. Cell suspensions of HB3 and Dd2 were incubated with 50 nM [3H]chloroquine and 25 nM to 50 μM nonradioactive chloroquine. The reactions were incubated at 4 °C, at which temperature chloroquine would bind to the transporter without being translocated (see Fig. 2). The amount of specifically bound chloroquine was determined and analyzed in a Woolf-Augustinsson-Hofstee plot. Linear regressions were observed for both HB3 and Dd2 (Fig. 5). The intercept on the y axis gives the number of chloroquine-binding sites, which was found to be the same for both HB3 and Dd2. The gradients, however, differ, indicating that HB3 and Dd2
have different binding constants for chloroquine, consistent with the data shown in Fig. 4. These data suggest that chloroquine resistance is correlated with kinetic changes in chloroquine uptake. The differences in $K_m$ and $J_{\text{max}}$ observed for Dd2 appear to result from an altered importer as opposed to a reduction in the number of facilitator molecules.

Genetic Linkage of Chloroquine Resistance with Kinetic Changes in Chloroquine Import—To date only two genetic crosses have been made with *P. falciparum*, of which one has used Dd2 and HB3 as parental clones (13). The analysis of the progeny from the HB3xDd2 cross provides us with an opportunity to investigate the possibility of a genetic linkage between the chloroquine-resistant phenotype and kinetic changes in chloroquine import. From this cross 16 independent progeny were investigated; 8 are CQS and 8 CQR. Apparent $K_m$ and maximal transport rate $J_{\text{max}}$ values were determined as described under “Experimental Procedures.”

Only two kinetic phenotypes were exhibited by the progeny (Fig. 6). For all 8 CQS progeny, the kinetics of chloroquine import were the same as those of the HB3 parental clone. Likewise, chloroquine import kinetics for the 8 CQR progeny were the same as those of the Dd2 parental clone. Thus, differences in the chloroquine import kinetics, indicated by altered apparent $K_m$ and apparent $J_{\text{max}}$ values, segregate with the chloroquine-resistant phenotype in a genetic cross.

**A Plasmodial Na\(^+\)/H\(^+\) Exchanger Mediates Chloroquine Import**—It has recently been shown in a bacterial system that chloroquine interacts with cation transporting systems (26). We explored this possibility in *P. falciparum* by examining the effect of substances inhibiting cation exchange on chloroquine uptake (data not shown). Compounds known to inhibit plasma membrane Na\(^+\)/H\(^+\) exchangers reversibly, such as amiloride and amiloride derivatives including 5-(N,N-dimethyl)amiloride and EIPA, reduced chloroquine uptake (data not shown). EIPA was chosen for further studies, due to its selective inhibition of...
eukaryotic Na\textsuperscript{+}/H\textsuperscript{+} exchangers (27–29), including that of \textit{P. falciparum} (30).

In both HB3 and Dd2, EIPA inhibits initial chloroquine uptake in a dose-dependent manner (Fig. 7a). Half-maximal inhibition is achieved at an EIPA concentration of about 6.2 ± 4.0 \textmu M for HB3 and 62 ± 20 \textmu M for Dd2. The fact that chloroquine uptake is inhibitable in infected but not uninfected erythrocytes provides further evidence for a \textit{P. falciparum}-encoded protein that mediates chloroquine import.

While it is possible that chloroquine import is mediated by a plasmodial Na\textsuperscript{+}/H\textsuperscript{+} exchanger, which is directly inhibited by EIPA, the data thus far presented cannot exclude the possibility of a secondary effect of EIPA on chloroquine uptake. To prove direct competition of EIPA and chloroquine for the same protein, more detailed inhibition studies were conducted. The initial velocity of chloroquine uptake by HB3 was monitored in the presence of three different EIPA concentrations. The data obtained were analyzed using a Lineweaver-Burk plot (Fig. 7b).

This analysis showed that inhibition of the initial chloroquine uptake by EIPA is strictly competitive. The apparent \( K_i \) is 6.6 \textmu M, as determined by plotting the slopes of the lines from Fig. 7b versus the EIPA concentration (data not shown). Thus, EIPA and chloroquine bind not only to the same protein but also seem to compete for the same binding site. Given the high specificity of EIPA, a plasma membrane Na\textsuperscript{+}/H\textsuperscript{+} exchanger appears to be responsible for chloroquine import in \textit{P. falciparum}.

**DISCUSSION**

Chloroquine is directed against the intra-erythrocytic \textit{P. falciparum} stages and at concentrations exceeding 0.1 mM inhibits the conversion of toxic heme into inert hemozoin (1, 2). Chloroquine resistance does not seem to involve changes in hemoglobin degradation, heme detoxification, or drug metabolism, but appears to be associated with the reduction of the intracellular chloroquine concentration below biologically effective levels (3, 5). The observation that CQR parasite isolates accumulate significantly less chloroquine than do CQS parasite isolates has aroused many speculations as to the molecular basis of this phenotype. Three different mechanisms are currently being considered: (i) the acquisition of a rapid chloroquine efflux mechanism (6, 7, 13); (ii) a rise in the lysosomal pH which would reduce acidotropic accumulation of chloroquine (14, 31); and (iii) the loss or reduction of a chloroquine influx mechanism (17).

As shown here, changes in the chloroquine import kinetics play a pivotal role in constituting the resistant phenotype. We have demonstrated that the initial chloroquine uptake is temperature-dependent, saturable, and inhibitable. These features are indicative of carrier-mediated transport and clearly demonstrate the existence of a chloroquine import mechanism in \textit{P. falciparum}. No evidence for such a chloroquine concentrating mechanism was found in uninfected erythrocytes, conforming to previous reports (24, 25). This suggests that the factor mediating chloroquine import is encoded by \textit{P. falciparum}.

Facilitated chloroquine import exists in both CQS and CQR parasite isolates, as shown for HB3 and Dd2, but has also been observed using other geographically dispersed CQS and CQR parasite isolates (data not shown). The kinetics of chloroquine import can be described using the Michaelis-Menten equation, as indicated by the hyperbolic relationship between the initial velocity of chloroquine uptake and the chloroquine concentration (Figs. 4 and 6). The kinetic parameters of this reaction were determined for both the CQS parasite clone HB3 and the CQR parasite clone Dd2 and found to differ. In HB3 the chloroquine import kinetics are defined by an apparent \( K_m \) of 16 ± 1.4 \textmu M and a \( J_{max} \) of 186 ± 20 fmol/1 × 10\textsuperscript{6} parasites/min. In

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**Fig. 7. Inhibition of initial [\textsuperscript{3}H]chloroquine uptake by EIPA. a, dose-response curve of EIPA on initial [\textsuperscript{3}H]chloroquine incorporation. The CQS \textit{P. falciparum} clone HB3 (●), the CQR clone Dd2 (○), and uninfected erythrocytes (△) were examined. The rates of initial [\textsuperscript{3}H]chloroquine uptake were determined over a range of EIPA concentrations. Chloroquine incorporation rates were calculated per 2 × 10\textsuperscript{7} erythrocytes, to allow comparison of infected with uninfected erythrocytes. Dd2 and HB3 cell cultures had a parasitemia of 4% trophozoites. Results represent the mean of three independent experiments. Inset details [\textsuperscript{3}H]chloroquine incorporation by Dd2. b, Lineweaver-Burk plot of the initial rate of [\textsuperscript{3}H]chloroquine incorporation as a function of the chloroquine concentration at different EIPA concentrations. Chloroquine uptake by the CQS \textit{P. falciparum} clone HB3 was examined. The EIPA concentrations used were: ○, 50 nM; ▼, 10 nM; △, 5 nM; □, 0 nM. The mean of three independent experiments is shown.**
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comparison, the CQR parasite clone Dd2 has a chloroquine transporter with an apparent \(K_m\) of 107 ± 17.6 nM and a \(J_{\text{max}}\) of 150 ± 20 fmol/1 × 10^6 parasites/min. Chloroquine binding assays confirmed the notion that Dd2 binds chloroquine with a lower affinity (Fig. 5).

Since a \(K_m\) value is an intrinsic property of a protein, the change in \(K_m\) for chloroquine suggests that the chloroquine importer is altered in Dd2. The number of chloroquine importers appears to be the same for both HB3 and Dd2. The 6.5-fold difference in affinity of the transporters for chloroquine can almost account for the 8-fold difference in chloroquine susceptibility observed between the two \(P. falciparum\) clones (13).

Investigation of chloroquine uptake by several other \(P. falciparum\) isolates confirmed the association of chloroquine resistance with changes in chloroquine import kinetics. CQR parasite isolates consistently have a transporter with a reduced transport rate and lower affinity for chloroquine (data not shown). Since sensitivity to chloroquine is clone-dependent, ranging from between 11 and 608 nM (12), it is tempting to speculate that the apparent \(K_m\) and \(J_{\text{max}}\) values of the chloroquine importer determine the degree of resistance.

Contrasting our findings, other studies have concluded that chloroquine uptake kinetics are identical in both CQR and CQS parasite isolates (7). However, these studies were conducted with chloroquine concentrations ranging from between 0.1 and 1 nM, which is several orders of magnitude below the apparent \(K_m\) values of the chloroquine importer, as determined in this study. Under these conditions chloroquine is quickly depleted from the media and becomes limiting, which results in a slow uptake in both CQS and CQR parasite isolates. According to our proposal, we predict that verapamil acts on chloroquine uptake in a dose-dependent manner (Fig. 7). Inhibition of chloroquine import by EIPA is strictly competitive, indicating that both EIPA and chloroquine bind to the same protein and compete for the same binding site. In other systems, the pharmacological action of EIPA has been demonstrated to be highly specific (27–29). At micromolar concentrations, EIPA inhibits the activity of eukaryotic Na+/H+ exchangers by competitively binding to, and blocking, the Na+- binding site (32–34). Binding of EIPA interferes with the biological function of a Na+/H+ exchanger in its role in the regulation of cytoplasmic pH and cellular volume (34).

Bosia et al. (30) have demonstrated the presence of an EIPA-inhibitable Na+/H+ exchanger in \(P. falciparum\), which resides within the parasite plasma membrane, relieving the cell of a surplus of protons generated during glycolysis. Inhibition of the plasmoidal Na+/H+ exchanger by EIPA resulted in an intracellular acidification (30).

Based on these findings, we conclude that chloroquine import is mediated by a \(P. falciparum\) Na+/H+ exchanger. Since both EIPA and chloroquine compete for the same binding site, it is plausible that chloroquine import occurs via the Na+-binding domain of the Na+/H+ exchanger, instead of sodium and in exchange of protons. Electrophysiological studies strengthen this proposal by demonstrating that chloroquine import is associated with an EIPA-inhibitable cytoplasmic alkalization.2

If chloroquine is transported in exchange of protons then one would expect to find a dependence of chloroquine resistance on the pH gradient across the parasite membrane. This is indeed found. Several studies have reported that changes in the transmembrane pH gradient provoke a change in the susceptibility of the parasite to chloroquine (18, 19, 35).

The activity of a Na+/H+ exchanger can be modulated by a broad range of factors, as shown in other systems (34). These include phosphorylation, hormone and growth factor induced binding of accessory proteins, and the binding of Ca2+/calmodulin (34). Several of these modulators not only affect the transport rate of a Na+/H+ exchanger but also its affinity for protons and possibly sodium (34). Thus, changes in the \(P. falciparum\) Na+/H+ exchanger’s affinity for chloroquine could result from several alternative scenarios: (i) a mutation of the active chloroquine-binding site; (ii) a conformational mutation elsewhere in the Na+/H+ exchanger, instead of sodium and in exchange of protons. Electrophysiological studies revealed that either a Na+/H+ exchanger or a modulating factor is mutated in response to chloroquine selection, electrophysiological studies revealed differences in cytoplasmic pH regulation of CQR and CQS parasite isolates.2

Interestingly, Martiney et al. (19) have recently shown that the Ca2+-channel blocker verapamil increased net chloroquine uptake in both CQS and CQR parasite isolates. According to our proposal, we predict that verapamil acts on chloroquine transport by modulating the Na+/H+ exchanger activity through the Ca2+/calmodulin regulatory pathway. We postulate that either a \(P. falciparum\) Na+/H+ exchanger or a factor regulating Na+/H+ exchanger activity resides within the chloroquine resistance locus, defined by the genetic cross between HB3 and Dd2 (36).

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