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Potential role of *Plasmodium falciparum* exported protein 1 in the chloroquine mode of action

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ABSTRACT

In the human malaria parasite *Plasmodium falciparum,* membrane glutathione *S*-transferases (GST) have recently emerged as potential cellular detoxifying units and as drug target candidates with the artemisinin (ART) class of antimalarials inhibiting their activity at single-digit nanomolar potency when activated by iron sources such as cytotoxic hematin. Here we put forward the hypothesis that the membrane GST *Plasmodium falciparum* exported protein 1 (*Pf*EXP1, PF3D7_1121600) might be directly involved in the mode of action of the unrelated antimalarial 4-aminoquinoline drug chloroquine (CQ). Along this line we report potent biochemical inhibition of membrane glutathione *S*-transferase activity in recombinant *Pf*EXP1 through CQ at half maximal inhibitory CQ concentrations of 9.02 nM and 19.33 nM when using hematin and the iron deficient 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, respectively. Thus, in contrast to ART, CQ may not require activation through an iron source such as hematin for a potent inhibition of membrane GST activity. Arguably, these data represent the first instance of low nanomolar inhibition of an essential *Plasmodium falciparum* enzyme through a 4-aminoquinoline and might encourage further investigation of *Pf*EXP1 as a potential CQ target candidate.

1. Introduction

The molecular and biochemical mechanisms of action for the 4-aminoquinoline chloroquine (CQ) remain to be fully understood, with one leading antimalarial effect depending on reducing the buildup of non-toxic hemozoin (Hz) biocrystals originating with the toxic heme that is liberated inside the malaria parasite's food vacuole (FV) upon catabolism of the host red blood cell (RBC) hemoglobin during the asexual blood stages of *Plasmodium falciparum* malaria (Ecker et al., 2012). However, even under normal conditions without CQ, a significant portion of heme, which upon liberation from hemoglobin inside the FV is rapidly oxidized to form cytotoxic hematin, evades conversion into hemozoin: estimates (Egan et al., 2002; Ginsburg et al., 1998) vary between 5% and 70% of the total heme/hematin stored at several mM concentrations inside the FV (Loria et al., 1999). But heme/hematin is long known to directly disrupt membrane structure and kill malaria parasites already at low micromolar concentrations (Orjih et al., 1981). Thus until it is shown that at any point during the asexual intraerythrocytic stages of malaria at least 99.9% of the free heme/hematin is readily transformed into Hz before its toxic and lethal effects on parasite membranes can take place other heme/hematin protection, sequestration or degradation mechanisms must be necessarily considered. For example, heme/hematin may also be degraded through a glutathione-dependent mechanism that complements Hz formation (Ecker et al., 2012; Ginsburg et al., 1998; Lisewski et al., 2014) in which initially the thiol group of reduced GSH spontaneously (i.e., without additional enzyme action) forms adducts with the iron inside the porphyrin ring (see, Fig. 1A for a model reaction). Such glutathione

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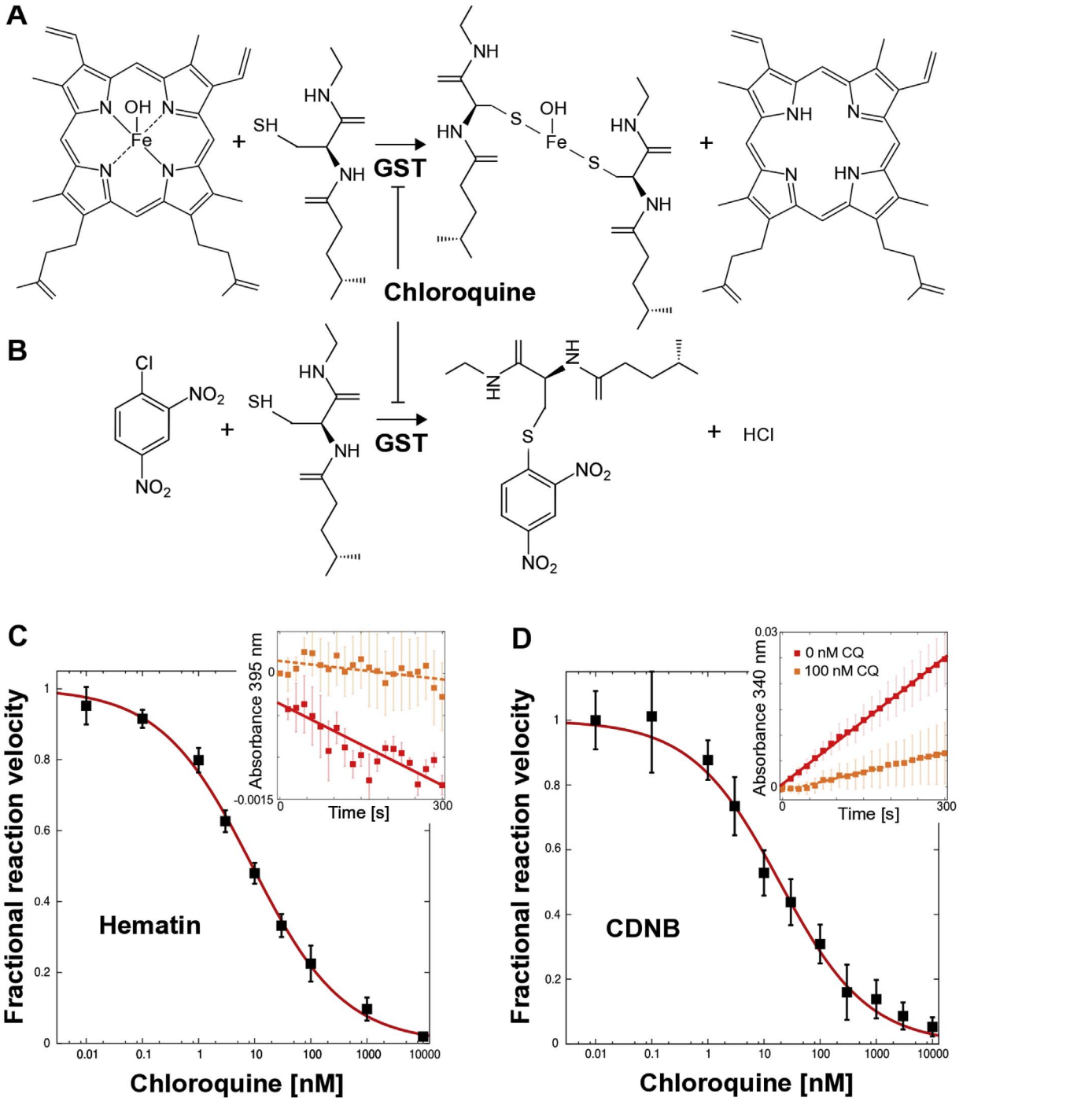

Fig. 1. Schematic overview of glutathione S-transferase (GST) mediated degradation reaction of the substrates hematin (A) and 1-chloro-2,4-dinitrobenzine (B). In (B), GST enzymatically generates a thiolate anion GS^- by proton removal from GSH. The thiolate then conjugates with CDNB, producing an intermediate Meisenheimer complex which, after chloride dissociation, gives the final glutathionyl-dinitrobenzene (GS-DNB) conjugate. In (A), a possible conjugate of a single glutathione molecule with the iron is not depicted. (C) CQ potently inhibits the GST activity of recombinant and bacterially (*E. coli*) expressed *Pf*EXP1 toward hematin with half maximal inhibitory concentration (IC_{50}) of 9.02 ± 0.69 nM and a Hill coefficient n_H = 0.53 ± 0.02. Inset: Inhibition kinetics from absorbance spectrophotometry at 395 nm at zero (red, solid line) and at 100 nM CQ concentration (orange, dashed line). (D) CQ also potently inhibits the GST activity of *Pf*EXP1 toward the standard GST substrate CDNB with an IC_{50} of 19.33 ± 2.67 nM and n_H = 0.51 ± 0.04. Inset: Inhibition kinetics from absorbance spectrophotometry at 340 nm at zero (red, solid line) and at 100 nM CQ concentration (orange, dashed line). The corresponding uninhibited (at 0 nM CQ concentration) GST specific activity toward CDNB was estimated at 7.3 ± 2.6 μmol/min/mg. Fractional velocities calculated from slopes to linear least square fits of the kinetics data. IC_{50} and Hill coefficient values n_H are least square fits to the standard dose response curve $1–(1 + 10^{nH\ (\log IC50 - \log [CQ])})^{-1}$. Error bars represent standard errors from at least three measurements. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mediated degradation pathways might also constitute potential antimalarial drug targets and play a role in drug resistance (Muller, 2015). We have recently identified (Lisewski et al., 2014) a more efficient enzymatic hematin reaction based on the functional characterization of the parasite's essential gene product *Plasmodium falciparum* exported protein 1 (*Pf*EXP1, also referred to as antigen 5.1, Ag5.1, or circumsporozoite related antigen, CRA, with gene identifier PF3D7_1121600). In contrast to the other known soluble, cytosolic *Plasmodium falciparum* glutathione *S*-transferase (*Pf*GST, PF3D7_1419300) the evolutionarily unrelated *Pf*EXP1 is from the parasitophorous vacuolar and early FV