Biochemical Pharmacology 97 (2015) 137–146

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Characterization of native PfABCG protein in *Plasmodium falciparum*

Sonia Edaye, Elias Georges*

Institute of Parasitology, McGill University, Ste. Anne de Bellevue, Quebec, Canada

ARTICLE INFO

Article history:
Received 20 March 2015
Accepted 29 June 2015
Available online 31 July 2015

Chemical compounds studied in this article:
Chloroquine diphosphate (CID: 64927)
Primaquine diphosphate (CID: 6135)
Ketotifen fumarate (CID: 5282408)

Keywords:
Malaria
Plasmodium falciparum
PfABCG
ABC transporter
Drug resistance
Ketotifen

ABSTRACT

The *Plasmodium falciparum* genome encodes 16 members of ABC proteins, with one member of the ABCG subfamily (PfABCG). Analysis of PfABCG amino acid sequence shows equal sequence identity to hsABCG1 and G2. Using N-terminal directed antibody against a recombinant fragment of PfABCG, we show that PfABCG migrates with an apparent molecular mass of 65KDa polypeptide on SDS-PAGE. PfABCG is expressed in all four stages of the parasite erythrocytic life cycle, with lower and higher expression in ring and late trophozoite stages, respectively. The protein localizes to the plasma membrane and a novel spherical structure beneath the cell membrane. Similar localization is also observed in gametocytes where PfABCG is highly expressed. Analysis of PfABCG genomic sequences for polymorphisms and changes in protein expression between different strains of *P. falciparum* revealed identical nucleotide sequence among the different strains, but variable protein expression. PfABCG expression is least in HB3 chloroquine sensitive strain, while higher expression levels are seen in other chloroquine-sensitive and -resistant strains, with highest levels of expression in 7G8. The differential expression of PfABCG in three chloroquine-sensitive strains (e.g., 3D7, HB3 and D10) predicts the sensitivity of the different strains to ketotifen, an anti-histaminic drug, whereby low expression is associated with decreased sensitivity to ketotifen. Taken together, the results in this report provide the first description of native PfABCG expression and subcellular localization in asexual stages of the parasite and its localization in gametocytes. It remains to be determined if PfABCG is functionally equivalent to mammalian ABCG1, ABCG2 or both.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Malaria remains one of the most deadly diseases affecting humanity with 665,000 to 1.2 million deaths annually [1,2]. In the absence of an effective and lasting vaccine, treatment for malaria infections continues to rely heavily on the use of several antimalarials that often leads to the rise and spread of drug resistant parasites. In the last two decades resistance to quinoline-based drugs has been attributed to the action of molecular efflux pumps that severely reduce the toxicity of such antimalarials. Two membrane transporters have been shown to cause resistance to antimalarials in *Plasmodium falciparum*: the chloroquine resistance transporter (PfCRT), a member of the drug metabolite transporters [3,4], and the P-glycoprotein homologue-1 (Pgh-1 or PfMDR1), a member of the ATP-binding cassette (ABC) proteins superfamily [5]. Although the normal substrates for PfCRT and PfMDR1 are not known, polymorphisms in these drug transporters have been shown to alter the parasite's response to Artemisinin-based Combination Therapy [6].

The ABC transporters constitute one of the largest families of proteins that are evolutionarily conserved from bacteria to human. Transmembrane ABC transporters encode at least one multiple spanning domain (MSD), with six transmembrane helices, and one cytoplasmic domain encoding an ATP-binding cassette [7]. Functionally, ABC transporters are organized either as full transporters containing two of each domain, or half transporters that homo- or hetero-dimerize to form a functionally active transporter [8]. In humans, the ABC transporter family consists of 48 members grouped into 7 subfamilies (ABCA to ABCG; [9]); while in *P. falciparum*, the ABC family consists of sixteen members grouped into six subfamilies (ABCB, C, E, F, G, and I; [10–12]). The B-subfamily of *P. falciparum* encodes seven proteins, with PfMDR1 and PfMDR2 (or PfABCB1 and PfABCB2, respectively) associated with drug resistance. Moreover, PfMDR1 shown to localize to the vacuolar membrane of the parasite digestive vacuole (DV) confers resistance to mefloquine and other related quinolines [13]. PfMDR2 is localized to the parasite plasma membrane and shown recently to confer resistance to heavy metals (e.g., cadmium) presumably through an efflux mechanism [14,15].

* Corresponding author at: Institute of Parasitology, Macdonald Campus, McGill University, Ste. Anne de Bellevue, Quebec H9X-3V9, Canada. Fax: + 1 514 398 7857.
E-mail address: Elias.Georges@McGill.CA (E. Georges).

http://dx.doi.org/10.1016/j.bcp.2015.06.035
0006-2952/© 2015 Elsevier Inc. All rights reserved.

Two additional ABC transporters, PfMRP1 and PfMRP2 (or PfABCC1 and PfABCC2), found at the parasite plasma membrane have been implicated in conferring resistance to quinoline drugs and the transport of glutathione [16,17]. The objective of this study was to characterize the expression of the only member of the G-subfamily (PfABCG) in different *P. falciparum* strains and to determine its subcellular localization in the parasite.

2. Materials and methods

2.1. Parasite cultures

P. falciparum strains (e.g., 3D7, D10, HB3, 7G8, K1 and W2) were obtained from the Reference Reagent Resource Center (MR4) (Manassas, VA, USA) and maintained in culture in RPMI-1640 medium supplemented with 25 mM HEPES, L-Glutamine (Life technologies Inc., Burlington, CA), 0.1 mM hypoxanthine (Sigma–Aldrich, Oakville, ON, CA) and 10 % heat-inactivated human plasma (Interstate blood bank, Manassas, USA). Parasites were allowed to proliferate on type A^+ human erythrocytes (Interstate Blood Bank, Manassas, USA) at 37 °C under conditions of 3% O_2, 5% CO_2 and 92% N_2. The parasite cultures were routinely synchronized with 5% Sorbitol (Sigma–Aldrich, Oakville, ON, CA) and parasitemia was assessed by blood smears stained with 5% Giemsa solution (Sigma–Aldrich, Oakville, ON, CA).

2.2. Recombinant expression and antibody production

The predicted DNA sequence encoding PfABCG (PF14_0244 or PF3D7_1426500) was retrieved from PlasmoDB database. PfABCG gene did not contain any introns hence; genomic DNA was used to amplify PfABCG coding sequence from 3D7 (Netherlands lab strain) genomic DNA. A fragment (1–1067 bp, encoding residues 1–356 of PfABCG N-terminal nucleotide binding domain ($PfABCG^{1-356}$)) was amplified with PfABCG-specific forward 5′ATGGATTTGAAAGGGTGGAT3′ and reverse 5′TCATTGTGTGGATAATATAGGTG3′ primers by PCR using Platinum Pfx DNA polymerase (Life technologies Inc., Burlington, ON, CA). The amplified DNA fragment was directionally cloned into pGEX-6P1 vector (Amersham Biosciences, Pittsburgh, PA, USA) at the C-terminal of GST protein and propagated in *E. coli*, strain BL21 (Life technologies inc, Burlington, ON, CA). GST-$PfABCG^{1-356}$ fusion protein was affinity purified on GSH-coupled Sepharose resin according to the manufacturer protocol (Amersham Biosciences, Pittsburgh, PA, USA), and subsequently used to generate polyclonal antibodies in rabbits (using McGill's Standard Operating Protocol; McGill Comparative Medicine and Animal Resources Center).

2.3. Parasite protein extraction and Western blotting

A parasite culture of 3D7 strain was synchronized at 5%–10% parasitemia and harvested at different stages of the parasite asexual life cycle: rings (6–12 hpi), trophozoites (20–26 hpi), late trophozoites (30–36 hpi) and schizonts (44 hpi) (hpi: hours post-infection). In addition to 3D7, other chloroquine-sensitive (D10, HB3) and -resistant parasite (7G8, K1, W2) cultures were synchronized and harvested at late trophozoites–schizonts stages by percoll gradient centrifugation as described by Victor Fernandez (Methods in Malaria Research, 5th edition, MR4, Manassas). Percoll purified parasites were harvested and washed twice in RPMI-1640, then resuspended in phosphate-buffered saline (PBS). The number of parasites or parasitized RBCs was determined using hemocytometer. The different stages and strains of parasites were released from infected RBCs by saponin treatment and the free parasites were lysed in PBS (pH 7) containing 0.25% sodium dodecyl sulfate (SDS) and 0.25% sodium deoxycholic acid (Sigma–Aldrich, Oakville, ON, CA), supplemented with Halt Protease Inhibitor Single-Use Cocktail (AEBSF 1 mM, Aprotinin 800 nM, Bestatin 5 μM, E64 15 μM, Leupeptin 20 μM, Pepstatin A 10 μM, EDTA 5 mM) (Thermo Scientific, Rockford, IL, USA). The erythrocytes were also treated with saponin and lysed with the same buffer. Lysates were cleared by centrifugation at 13,000 rpm at 4 °C for 10 min. Parasites lysates were mixed with 2X SDS PAGE sample loading buffer and resolved onto 8% acrylamide SDS PAGE. Resolved proteins were transferred to PVDF membranes and probed with PfABCG antiserum (dilution, 1:1000) and horseradish peroxidase-conjugated goat anti-rabbit IgG (dilution, 1:5000 v/v). The reactive polypeptide bands were visualized using the SuperSignal West Pico chemiluminescent kit according to the manufacturer's protocol (Thermo Scientific, Rockford, IL, USA).

2.4. RNA extraction and Real time PCR

P. falciparum 3D7, HB3 and 7G8 parasite were grown, synchronized 6–12 h post-invasion and collected 24 h after synchronization. Total RNA was extracted with TriZol reagent (Life technologies Inc., Burlington, ON, CA) and quantified using NanoDrop™ 1000 (Thermo Scientific, Rockford, IL, USA). RNA purity was confirmed using PCR amplification of an intron-spanning region of PfCRT gene. Aliquots of purified RNA (1 μg) from different strains of *P. falciparum* were reverse transcribed using QuantiTect reverse transcription kit (QIAGEN, Toronto, ON, CA). The resultant cDNAs were compared by real time PCR using the Power SYBR® Green PCR master mix (Life technologies Inc., Burlington, ON, CA). The relative expression of PfABCG transcript in the different strains relative to the reference strain (3D7) was calculated using the $2^{-\Delta DCt}$ method [18]. The seryl-tRNA synthetase (PF3D7_0717700) gene was used as the internal reference gene [19,20]. The nucleotide sequences of all qPCR primers used above are shown in Table 2.

2.5. Immunofluorescence staining

Parasitized erythrocytes were washed once with PBS and then fixed with 4% paraformaldehyde (Sigma–Aldrich, Oakville, ON, CA) in PBS for 30 min at room temperature. Fixed cells were washed once in PBS and then applied onto poly-L-lysine coated slide cover slips. Slides were air dried for 5 min and quenched with 0.15% glycine (Sigma–Aldrich, Oakville, ON, CA) in PBS for 10 min at room temperature. Slides were subjected to a wash step with PBS-0.05% Tween20 (PBST) and then permeabilized with 0.1% tritonX-100 (Sigma–Aldrich, Oakville, ON, CA) in PBS for 10 min at room temperature. After washing three times with PBST, cells were blocked with 1% normal goat serum in PBST for 1hr at room temperature. Cover slips were then incubated with PfABCG antiserum (1:50 v/v dilution) at 4 °C overnight followed by three 10 min washes with PBST. Alexa-fluor 488-conjugated goat anti-rabbit antibody (Life technologies Inc., Burlington, ON, CA) was added at 1:2000 dilution for 45 min. For the double immunohistochemical staining of infected erythrocytes, anti-PfEXP-1 rabbit antibody (kind gift from Dr. Rohrbach) was used at 1:100 v/v dilution, followed by an incubation with Alexa-fluor 594-conjugated goat anti-rabbit second antibody (Life technologies Inc., Burlington, ON, CA) (1:2000 v/v). After three washes with PBST, the coverslips were incubated with anti-PfABCG or anti-PfCRT rabbit antibody (Baakdah F. and Georges E., Unpublished results) at 1:50 v/v dilution and subsequently with Alexa-fluor 488-conjugated goat anti-rabbit second antibody (1:2000 v/v). Control experiments showed cross-reactive staining or signal when Alexa-fluor 488-conjugated goat anti-rabbit second antibody was added (results not shown). Cover slips were rinsed three times with PBST, incubated for 5 min with DAPI (1 μg/ml) and washed further with PBST. The cover slips were mounted on microscope slides using

Download English Version:

https://daneshyari.com/en/article/2511972

Download Persian Version:

https://daneshyari.com/article/2511972

Daneshyari.com