Parasitol Res (2008) 102:805–807
DOI 10.1007/s00436-007-0836-9

SHORT COMMUNICATION

Inhibition of glutathione-*S*-transferase from *Plasmodium yoelii* by protoporphyrin IX, cibacron blue and menadione: implications and therapeutic benefits

Rumana Ahmad · Arvind K. Srivastava

Received: 29 August 2007 / Accepted: 30 November 2007 / Published online: 5 January 2008
© Springer-Verlag 2007

Abstract The rapidly developing resistance to drugs used for prophylaxis and treatment of malaria makes the identification of novel drug targets necessary. Glutathione-*S*-transferase (GST, E.C. 2.5.1.18), an important enzyme of the glutathione (GSH) cycle, is considered to be an essential detoxification enzyme in malarial parasites. Selective inhibition of this enzyme from malarial parasites by various classes of inhibitors may be viewed as a potential chemotherapeutic strategy to combat malaria. Purified GST from *Plasmodium yoelii* was inhibited by compounds like protoporphyrin IX, cibacron blue, as well as by the GSH depletor menadione. Cytosolic GST was inhibited to varying degrees by each compound. A characteristic inhibitor constant (K_i) was obtained for each inhibitor. The possible consequences of selective inhibition of parasitic GST to that of the host are discussed in relation to the chemotherapy of malaria.

Introduction

The emergence of strains resistant to drugs used for prophylaxis and treatment of malaria with presently no vaccine available has contributed to the spread and growth of malaria to various tropical and subtropical regions of the world. Sophisticated defence strategies have evolved in parasitic organisms that enable them to deal with a broad range of foreign and endogenously derived toxic compounds. Most of these structurally diverse, non-polar molecules instead of undergoing modification for their utilisation are detoxified and eliminated by a phase II detoxification enzyme known as glutathione-*S*-transferase (GST, E.C. 2.5.1.18) which is found in most parasites (Jakoby and Ziegler 1990).

Native GST from *Plasmodium yoelii* has been purified and characterised (Ahmad and Srivastava 2007a), and GST of *P. falciparum* (*Pf*-GST1) has been recombinantly expressed and a substrate as well as inhibitor profiling has also been carried out on the recombinant protein (Harwaldt et al. 2002; Liebau et al. 2002). The enzyme exhibits moderate non-selenium-dependent glutathione peroxidase (GPX) activity and potentially contributes to the protection of the parasite during oxidative stress situations in the parasitised red blood cells. Consequently, it is thereby hypothesised that GST inhibitors would have antimalarial effects per se by preventing parasite resistance against chloroquine (Harwaldt et al. 2002).

Although the utility of this enzyme as a drug target in effectively combating malaria is yet to be established, the critical role played by *Pf*-GST1 in detoxification makes this enzyme a viable drug target against malaria. The present study investigates the relative susceptibility of GST from rodent malarial parasites *P. yoelii* to inhibition by various 'non-substrate ligands' such as protoporphyrin IX, cibacron blue and menadione (Md), which exhibit non-competitive inhibition towards GST. The data reported here have the potential to form the basis of structure-based design of selective inhibitors, which may serve as antimalarial drug leads.

Materials and methods

Chemicals

Cibacron blue, protoporphyrin IX and menadione sodium bisulfite were purchased from Sigma Chemical Co., USA.

R. Ahmad · A. K. Srivastava (✉)
Division of Biochemistry, Central Drug Research Institute,
Chattar Manzil Palace,
P.O. Box No. 173, Lucknow 226001, India
e-mail: drarv1955@yahoo.com

All chemicals used in the isolation of malarial parasites, preparation, activity determination and purification of GST were obtained as mentioned in Ahmad and Srivastava (2007a).

P. yoelii nigeriensis infection in Swiss albino mice

In vivo maintenance and isolation of malarial parasites *P. yoelii* was carried out according to Ahmad and Srivastava (2007a).

Preparation of GST from malarial parasites *P. yoelii nigeriensis*

GST was prepared from malarial parasites *P. yoelii* as reported earlier (Ahmad and Srivastava 2007a), and GST activity was determined following the method of Habig et al. (1974). Protein was estimated by the method of Lowry et al. (1951) using BSA as standard.

Purification of GST from *P. yoelii*

GST was purified from the cytosolic fraction of *P. yoelii* by the method published earlier (Ahmad and Srivastava 2007a).

Inhibition studies

In order to determine the dose-dependent effect of various inhibitors and their respective inhibitor constants (K_i), the enzyme was incubated with varying concentrations of inhibitor for 10 min at room temperature in the presence of 100 mM potassium phosphate buffer and 1.0 mM glutathione (GSH). Reaction was initiated by the addition of 1.0 mM CDNB, and the absorbance at 340 nm was monitored for 5 min at 30-s intervals. The percentage inhibition of the enzyme activity by various inhibitors was calculated by comparing with a control tube. Results were expressed as mean±S.D. based on experiments done in quadruplicates.

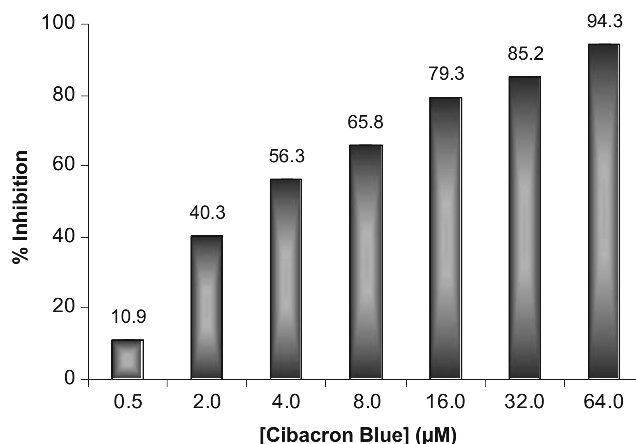

Fig. 2 Inhibition profile of *P. yoelii* GST by cibacron blue. Results were expressed as mean±S.D. based on experiments done in quadruplicates

Results

Effect of protoporphyrin IX on GST activity from *P. yoelii*

Protoporphyrin IX caused a concentration-dependent inhibition when studied for its effect in the concentration range of 0.25 to 200 μM (Fig. 1). It inhibited GST to the tune of 31.3% to 85.6% with an apparent K_i of around 13 μM.

Effect of cibacron blue on GST activity from *P. yoelii*

The dye cibacron blue also exhibited a concentration-dependent inhibition profile when studied for its effect in the concentration range of 0.5 to 64 μM (Fig. 2). It inhibited GST to the tune of 94.3% at 64 μM concentration with an apparent K_i of around 0.4 μM.

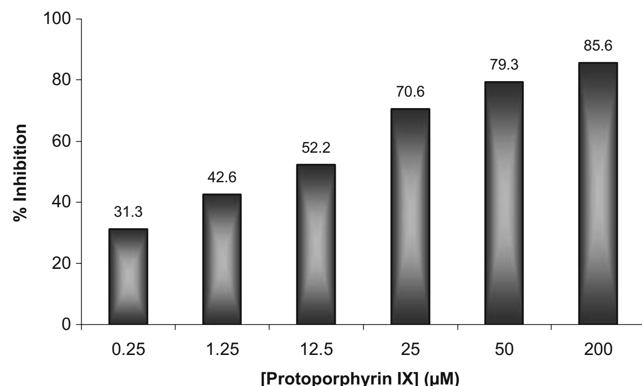

Fig. 1 Inhibition profile of *P. yoelii* GST by protoporphyrin IX. Results were expressed as mean±S.D. based on experiments done in quadruplicates

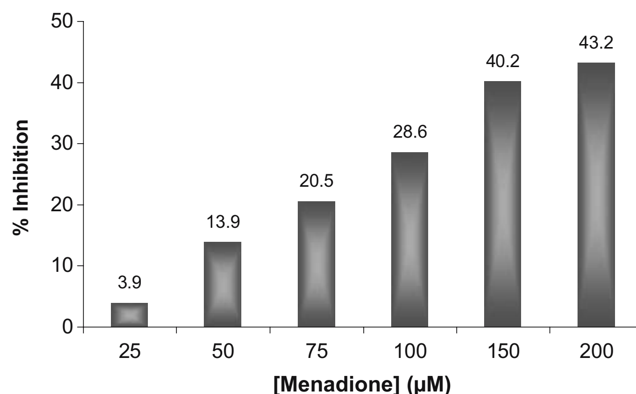

Fig. 3 Inhibition profile of *P. yoelii* GST by menadione. Results were expressed as mean±S.D. based on experiments done in quadruplicates

Effect of menadione on GST activity from *P. yoelii*

Menadione (2-methyl-1, 4-naphthoquinone, vitamin K), a known inhibitor of mammalian glutamate cysteine ligase and glutathione reductase, has also been used to prevent vitamin K deficiencies, as well as to treat malaria (Ahmad and Srivastava 2007b). Md was found to inhibit *P. yoelii* GST activity when studied for its effect in the concentration range of 25 to 200 μM (Fig. 3). It inhibited GST to the tune of 3.9% to 43.2% with an apparent K_i of around 80 μM.

Discussion

In the past decade, there have been great advances in our understanding of the biology of parasites. Recent discoveries regarding the physiology and biochemistry of protozoan and helminth parasites have elucidated many of the prospective targets that are unique to each parasite. Knowledge of the biochemical and molecular differences between the parasite and host and of the rate limiting points in the parasite metabolism are both necessary for the successful selection of prospective targets. Differences between the parasite and host enzymes may include differential sensitivity to inhibitors (Mansour 2002).

Ferriprotoporphyrin IX chloride (hemin), a known inhibitor of mammalian GST, has been demonstrated to exhibit non-competitive inhibition kinetics with respect to substrate GSH (Ahmad and Srivastava 2007a). Protoporphyrin IX and other tetrapyrroles are bound non-covalently ('liganded') to GST but not conjugated with reduced glutathione. The IC_{50} values, i.e. the concentrations of the inhibitors at which there is approximately 50% inhibition in the activity of the enzyme for protoporphyrin IX (12.5 μM), cibacron blue (56.3 μM) and menadione (>200 μM) for *P. yoelii* GST, were significantly different from those reported for GST from human placenta (Harwaldt et al. 2002).

The present study reports the *in vitro* effects of three compounds on malarial GST. Several other synthetic compounds belonging to various chemical classes showing *in vitro* GST inhibitory/modulatory activity have also been identified by us (Ahmad et al. 2007). A few of these compounds have been found to selectively inhibit the parasitic enzyme without affecting the activity of the host (mammalian) enzyme. *In vivo* experiments using Swiss albino mice are being carried on such compounds for proving the validity of the GST as a target for antimalarial drugs. It remains to be seen which of these compounds inhibit/modulate the activity of *P. yoelii* GST *in vivo* as well as the mechanism of inhibition. Inhibition of malarial GST is furthermore expected to act at different vulnerable metabolic sites of the parasite. The lack of functional *Plasmodium* GST is likely to disturb GSH-dependent detoxification processes, to enhance the levels of cytotoxic peroxides and possibly to increase the concentration of toxic hemin. With the *Pf*-GST structure and the features of the enzyme at hand, it might be possible to open new avenues for the development of novel antimalarial drugs and of drugs that help in antagonising chloroquine resistance.

Acknowledgements Financial assistance from the Council for Scientific and Industrial Research (CSIR), New Delhi (India) in the form of Senior Research Fellowship to R. Ahmad and Volkswagen Stiftung, Hannover (Germany) in the form of ad hoc research grant to A.K. Srivastava is gratefully acknowledged.

References

Ahmad R, Srivastava AK (2007a) Purification and biochemical characterization of cytosolic glutathione-*S*-transferase from malarial parasites *Plasmodium yoelii*. Parasitol Res 100:581–588

Ahmad R, Srivastava AK (2007b) Effect of malaria infection on hepatic and splenic glutathione-*S*-transferase(s) in Swiss albino and db/+ mice: efficacy of mefloquine and menadione in antimalarial chemotherapy. Parasitology 134:931–938

Ahmad R, Srivastava AK, Tripathi RP, Batra S, Walter RD (2007) Synthesis and biological evaluation of potential modulators of malarial glutathione-*S*-transferase. J Enzyme Inhib Med Chem 22:327–342

Habig HW, Pabst MJ, Jakoby WB (1974) Glutathione-*S*-transferase (s): the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Harwaldt P, Rahlfs S, Becker K (2002) Glutathione-*S*-transferase of the malarial parasite *Plasmodium falciparum*: characterization of a potential drug target. Biol Chem 383:821–830

Jakoby WB, Ziegler DM (1990) The enzymes of detoxication. J Biol Chem 265:20715–20718

Liebau E, Bergmann B, Campbell AM, Teesdale-Spittle P, Brophy PM, Lüersen K, Walter RD (2002) The glutathione-*S*-transferase from *Plasmodium falciparum*. Mol Biochem Parasitol 124:85–90

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–276

Mansour TE (2002) The search for antiparasitic agents. In: Chemotherapeutic targets in parasites: contemporary strategies. Cambridge University Press, United Kingdom, pp 1–16