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Purification and kinetic analysis of cytosolic and mitochondrial thioredoxin glutathione reductase extracted from *Taenia solium* cysticerci

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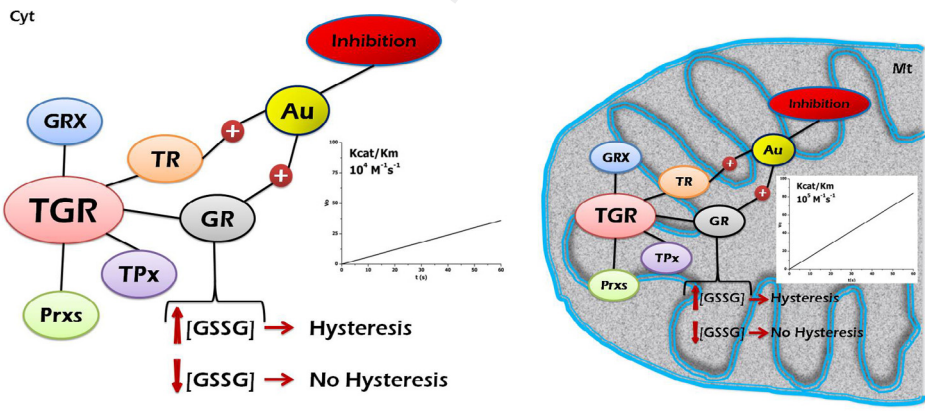
HIGHLIGHTS

- Thioredoxin glutathione reductases (TGRs) from *Taenia solium* tissues were purified.
- *T. solium* TGRs are multifunctional enzymes as classic TGRs described.
- Hysteretic kinetic properties showed both cytoplasm and mitochondrial *T. solium* TGRs.
- *T. solium* TGRs as selenoproteins were inhibited by nanomolar amounts of auranofin.
- Thioredoxin and glutathione systems are now demonstrated simultaneously in *T. solium*.

GRAPHICAL ABSTRACT



Cyt – citosolic, Mt – mitochondrial, Grx- Glutaredoxin, Prxs – Peroxiredoxin, TPx – Thioredoxin peroxidase, Tr – Thioredoxin reductase, GR – Glutathione reductase, Au - auranofin

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ABSTRACT

Thioredoxin glutathione reductases (TGRs) (EC 1.8.1.9) were purified to homogeneity from the cytosolic (cTsTGR) and mitochondrial (mTsTGR) fractions of *Taenia solium*, the agent responsible for neurocysticercosis, one of the major central nervous system parasitic diseases in humans. TsTGRs had a relative molecular weight of 132,000, while the corresponding value per subunit obtained under denaturing conditions, was of 62,000. Specific activities for thioredoxin reductase and glutathione reductase substrates for both TGRs explored were in the range or lower than values obtained for other platyhelminths and mammalian TGRs. cTsTGR and mTsTGR also showed hydroperoxide reductase activity using hydroperoxide as substrate. $K_{m(DTNB)}$ and $K_{cat(DTNB)}$ values for cTsTGR and mTsTGR (88 μM and 1.9 s^{-1}; 45 μM and 12.6 s^{-1}, respectively) and $K_{m(GSSG)}$ and $K_{cat(GSSG)}$ values for cTsTGR and mTsTGR (6.3 μM and 0.96 s^{-1}; 4 μM and 1.62 s^{-1}, respectively) were similar to or lower than those reported for mammalian TGRs. Mass spectrometry analysis showed that 12 peptides from cTsTGR and seven from mTsTGR were a match for gi|29825896 thioredoxin glutathione reductase [*Echinococcus granulosus*], confirming that both enzymes are TGRs. Both *T. solium* TGRs were inhibited by the gold compound auranofin, a selective inhibitor of thiol-dependent flavoreductases (I_{50} = 3.25, 2.29 nM for DTNB and GSSG substrates, respectively for cTsTGR; I_{50} = 5.6, 25.4 nM for mTsTGR

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toward the same substrates in the described order). Glutathione reductase activity of cTsTGR and mTsTGR exhibited hysteretic behavior with moderate to high concentrations of GSSG; this result was not observed either with thioredoxin, DTNB or NADPH. However, the observed hysteretic kinetics was suppressed with increasing amounts of both parasitic TGRs. These data suggest the existence of an effective substitute which may account for the lack of the detoxification enzymes glutathione reductase and thioredoxin reductase in *T. solium*, as has been described for very few other platyhelminths.

1. Introduction

Neurocysticercosis (NCC), a condition arising from the larval stage of *Taenia solium* in the central nervous system (CNS) of humans, is a neurological complex parasitic disease which may or may not yield symptoms in infected human hosts (Carpio, 2002). Symptoms include a severe immunological reaction developed by polymorfonuclear cells surrounding the metacestodes and with the ability to destroy them. When symptoms are absent, a few inflammatory cells may be observed in the vicinity of the unaffected parasite tissues.

In NCC the production of toxic lipid peroxidation metabolites appears as a consequence of inflammatory cell activation by *T. solium* cysticerci antigens (Comporti, 1993; Sotelo and Del Bruto, 2002). Patients with NCC exhibit high lipid peroxidation products in their cerebrospinal fluid (CSF) related to neurological symptoms (Rodriguez et al., 2008), a condition which is absent in asymptomatic patients lodging cysticerci in their CNS. The existence of the latter condition despite long-term infection (Dixon and Lipscomb, 1961), suggests that the parasite not only behaves as a passive onlooker during infection but that it also contributes to disrupt the expression of host resistance carried out by lipid peroxidation metabolites. Consequently, it appears that *T. solium* must possess adequate detoxification mechanisms, as may be concluded from the existence of asymptomatic carriers.

In mammalian tissue, antioxidant defenses against lipid peroxidation metabolites rely on two major independent pathways: the glutathione (GSH) and the thioredoxin (Trx) systems (Fernandes and Holmgren, 2004; Winyard et al., 2005). The flavoenzyme thioredoxin reductase (TrxR) constitutes the enzyme center in the Trx system given its ability to accept reducing equivalents from NADPH and to transfer them to Trx, which in turn, may reduce the detoxification enzymes peroxiredoxins (Pxs) that reduce H_2O_2 producing H_2O. TrxR is characterized by the GCUG motif at the C terminus, where U indicates selenocysteine (Sec) which is essential for its activity and function. Glutathione reductase (GR) is the corresponding enzyme center of the GSH system, given its ability to transfer electrons from NADPH to oxidized glutathione (GSSG), which results in the formation of two GSH molecules. Glutathione, in turn, may transfer electrons to oxidized dithiol and glutaredoxin (Grx), a small thiol-disulfide oxidoreductase capable of reducing several different targets. Peroxiredoxin and glutathione transferases, also detoxification enzymes, accept electrons proceeding respectively from the Trx and GSH pathways, and thus reduce H_2O_2 and other organic peroxides which constitute the source of lipid peroxidation.

At present, specialized TrxR and GR in *T. solium* have not been detected, and it may be useful to bear in mind that TrxR and GR enzymes in *Schistosoma mansoni* and other platyhelminths have not been isolated as independent entities (Alger and Williams, 2002; Bonilla et al., 2008). Instead of those proteins, some platyhelminths have a GR and TrxR molecular link exhibiting the fusion of glutaredoxin (Grx) and thioredoxin reductase (TrxR) domains into a single protein, a selenocysteine-containing enzyme which acts as thioredoxin glutathione reductase (TGR) (Salinas et al., 2004; Sun et al., 2001). TGR thus plays a central role in thiol-disulfide redox reactions by providing electrons to essential detoxification enzymes such as GR and Prx. GR reduces the tripeptide GSSG to GSH, which acts as main reducing agent in the catalytic functions displayed by GSTs (Mannervik and Danielson, 1988). It has also been shown that the most abundant GST in *T. solium* (Ts26GST) catalyzes lipid peroxidation (Plancarte et al., 2004) and that a *T. solium* peroxiredoxin (Ts2-CysPrx) was able to inactivate H_2O_2 and cummene hydroperoxide (Molina-López et al., 2006), most likely due to the presence of a Trx system present in *T. solium* tissues. In order to arrive at a better understanding of the detoxification mechanism exhibited by *T. solium*, we decided to investigate the presence of TGRs in this parasite's tissues.

2. Materials and methods

2.1. Materials

Chromatography resins were from the following source: DEAE-sepharose and 2′5′ADP-sepharose 4B from GE Healthcare Co. (Fairfield, CT, USA). NADPH, GSH, GSSG, 2-hydroxyethyl disulfide (HED), *Escherichia coli* and thioredoxin were from Aldrich Chemical Company (Milwaukee, WI, USA); Amicon PM 10 membrane from Diaflo, (Danvers, MA, USA); polyvinylidene difluoride (PVDF) from Millipore Corporation, (Billerica, MA, USA). Auranofin was purchased from ICN Biomedicals Inc. (Santa Ana, CA, USA). All other chemicals were reagent grade and obtained from common commercial sources.

2.2. Parasites

Taenia solium cysticerci were obtained from naturally infected cysticercotic pigs from the states of Morelos and Guerrero in Mexico. Parasites were dissected from their skeletal muscle and washed thoroughly with 150 mM NaCl, partially dried in filter paper, and immediately frozen at –70 °C until used, taking special care to avoid host tissues.

2.3. Isolation of the cytosolic protein fraction (CPF)

Taenia solium TGRs were purified by using a modified procedure previously developed by Sun et al. (2001). Briefly, *T. solium* cysticerci (15 g) were homogenized in one and a half volume of working buffer made of 50 mM imidazole-HCl (pH 6.5), containing 1 mM EDTA and 0.1 mM phenylmethyl-sulfonyl-fluoride (PMSF), using a Polytron homogenizer (Brinkmann Instruments). The homogenate containing 35 mg·ml^{-1} was spun 20 minutes at 10,000 g_{av}, the supernatant thus obtained was spun again for 1 h at 105,000 g_{av} to obtain the CPF.

2.4. Isolation of the soluble mitochondrial matrix proteins (SMMP)

Purification of *T. solium* mitochondrial fraction (MF) was done at 4 °C by differential centrifugation procedures as described by Ernest et al. (1962) with some modifications. Briefly, 15 g of parasites were homogenized in 15 ml of working buffer with 1 mM EGTA and 0.25 M sucrose (WBES), using a Polytron homogenizer (Brinkmann Instruments, Inc. Westbury, NY, USA). The homogenate with 9 mg·ml^{-1} was spun 20 minutes at 180 g_{av} and the supernatant 1 (Sn1) was kept aside. Pellet 1 (P1) (detritus

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