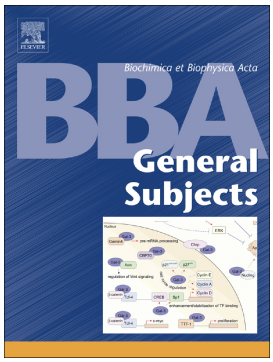
Accepted Manuscript



Biochemical and thermodynamic comparison of the selenocysteine containing and non-containing thioredoxin glutathione reductase of Fasciola gigantica

Parismita Kalita, Harish Shukla, Rohit Shukla, Timir Tripathi

PII: S0304-4165(18)30063-1
DOI: doi:10.1016/j.bbagen.2018.03.007
Reference: BBAGEN 29058

To appear in:

Received date: 14 November 2017
Revised date: 2 March 2018
Accepted date: 6 March 2018

Please cite this article as: Parismita Kalita, Harish Shukla, Rohit Shukla, Timir Tripathi , Biochemical and thermodynamic comparison of the selenocysteine containing and non-containing thioredoxin glutathione reductase of Fasciola gigantica. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Bbagen(2018), doi:10.1016/j.bbagen.2018.03.007

Biochemical and thermodynamic comparison of the selenocysteine containing and non-containing thioredoxin glutathione reductase of *Fasciola gigantica*

Parismita Kalita, Harish Shukla, Rohit Shukla, and Timir Tripathi*

Molecular and Structural Biophysics Laboratory, Department of Biochemistry, North-Eastern Hill University, Shillong 793022, India

Running title: Comparison of FgTGRsec and FgTGR

*To whom correspondence should be addressed: Dr. Timir Tripathi, Department of Biochemistry, North-Eastern Hill University, Shillong- 793022, India. Email: timir.tripathi@gmail.com, ttripathi@nehu.ac.in. Tel: +91-364-2722141; Fax: +91-364-2550108.

ABSTRACT

The thiol-disulfide redox metabolism in platyhelminth parasites depends entirely on a single selenocysteine (Sec) containing flavoenzyme, thioredoxin glutathione reductase (TGR) that links the classical thioredoxin (Trx) and glutathione (GSH) systems. In the present study, we investigated the catalytic and structural properties of different variants of *Fasciola gigantica* TGR to understand the role of Sec. The recombinant full-length Sec containing TGR (FgTGRsec), TGR without Sec (FgTGR) and TGRsec without the N-terminal glutaredoxin (Grx) domain (ΔNTD-FgTGRsec) were purified to homogeneity. Biochemical studies revealed that Sec597 is responsible for higher thioredoxin reductase (TrxR) and glutathione reductase (GR) activity of FgTGRsec. The N-terminal Grx domain was found to positively regulate the DTNB-based TrxR activity of FgTGRsec. The FgTGRsec was highly sensitive to inhibition by auranofin (AF). The structure of FgTGR was modeled, and the inhibitor AF was docked, and binding sites were identified. Unfolding studies suggest that all three proteins are highly cooperative molecules since during GdnHCl-induced denaturation, a monophasic unfolding of the proteins without stabilization of any intermediate is observed. The *C*m for GdnHCl induced unfolding of FgTGR was higher than FgTGRsec and ΔNTD-FgTGRsec suggesting that FgTGR without Sec was more stable in solution than the other protein variants. The free energy of