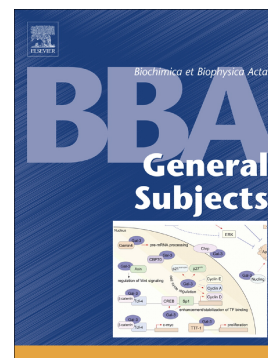


## Accepted Manuscript

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PII: S0304-4165(18)30063-1  
DOI: doi:[10.1016/j.bbagen.2018.03.007](https://doi.org/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](https://doi.org/10.1016/j.bbagen.2018.03.007)

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

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*Running title: Comparison of FgTGRsec and FgTGR*

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**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 ( $\Delta$ NTD-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  $\Delta$ NTD-FgTGRsec suggesting that FgTGR without Sec was more stable in solution than the other protein variants. The free energy of

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