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BIOCHEMICAL & BIOPHYSICAL CHARACTERIZATION AND THERMODYNAMIC COMPARISON OF DIFFERENT VARIANTS OF THE REDOX SELENOZYME THIOREDOXIN GLUTATHIONE REDUCTASE OF *FASCIOLA GIGANTICA*

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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, 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 Cm 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 stabilization for the proteins was also determined. To our knowledge, this is also the first report on unfolding and stability analysis of any TGR.