Engineering a Pseudo-26-kDa *Schistosoma* Glutathione Transferase from *bovis*/*haematobium* for Structure, Kinetics, and Ligandin Studies.

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Glutathione transferases (GSTs) are the main detoxification enzymes in schistosomes (Alger and Williams, 2002). These parasitic enzymes tend to be upregulated during drug treatment, with *Schistosoma haematobium* being one of the species that mainly affect humans. There is a lack of complete sequence information on the closely related *bovis* and *haematobium* 26-kDa GST isoforms in any database (Hsu *et al.*, 1966; Agnew *et al.*, 1989; Huyse *et al.*, 2009). Consequently, we engineered a pseudo-26-kDa S. *bovis*/*haematobium* GST (Sbh26GST) to understand structure–function relations and ligandin activity towards selected potential ligands. Sbh26GST was overexpressed in *Escherichia coli* as an MBP-fusion protein, purified to homogeneity and catalysed 1-chloro-2,4-dinitrobenzene-glutathione (CDNB-GSH) conjugation activity, with a specific activity of 13 µmol/min/mg. This activity decreased by ~95% in the presence of bromosulfophthalein (BSP), which showed an IC_{50} of 27 µM. Additionally, enzyme kinetics revealed that BSP acts as a non-competitive inhibitor relative to GSH. Spectroscopic studies affirmed that Sbh26GST adopts the canonical GST structure, which is predominantly α-helical. Further extrinsic 8-anilino-1-naphthalenesulfonate (ANS) spectroscopy illustrated that BSP, praziquantel (PZQ), and artemisinin (ART) might preferentially bind at the dimer interface or in proximity to the hydrophobic substrate-binding site of the enzyme. The Sbh26GST-BSP interaction is both enthalpically and entropically driven, with a stoichiometry of one BSP molecule per Sbh26GST dimer. Enzyme stability appeared enhanced in the presence of BSP and GSH. Induced fit ligand docking affirmed the spectroscopic, thermodynamic, and molecular modelling results. In conclusion, BSP is a potent inhibitor of Sbh26GST and could potentially be rationalized as a treatment for schistosomiasis.

Keywords: *Schistosoma*; glutathione transferase; praziquantel; CDNB assay; kinetics; isothermal titration calorimetry; thermal shift assay; spectroscopy; inhibition; bromosulfophthalein

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