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Comprehensive analysis of the catalytic and structural properties of a mu-class glutathione s-transferase from Fasciola Gigantica

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Abstract

Glutathione S-transferases (GSTs) play an important role in the detoxification of xenobiotics. They catalyze the nucleophilic addition of glutathione (GSH) to nonpolar compounds, rendering the products watersoluble. Fascioliasis is a neglected tropical disease caused by the foodborne trematodes Fasciola hepatica and Fasciola gigantica. These parasites infect mammals through ingestion of aquatic plants or contaminated water having encysted metacercaria. GST plays important roles in maintaining the cellular homeostasis, protection against oxidative stress and detoxification of xenobiotics thereby helping in survival. In the present study, we have investigated the catalytic and structural properties of a mu-class GST from the liver Fasciola gigantica (FgGST1). This will help in understanding the structure-function relationship of GSTs in these flukes. The gst1 gene was amplified, cloned in pET23a vector and overexpressed in BL21(DE3) cells. The purified recombinant FgGST1 formed a homodimer and composed of ~25 kDa subunit. Kinetic analysis revealed that FgGST1 displays broad substrate specificity and shows high GSH conjugation activity towards 1-chloro-2,4- dinitrobenzene, 4-nitroquinoline-1-oxide, trans-4-phenyl-3-butene-2-one and peroxidase activity towards trans-2-nonenal and hexa-2,4-dienal. The FgGST1 was highly sensitive to inhibition by Cibacron blue. The cofactor(GSH) and inhibitor(Cibacron blue) were docked against FgGST1 and binding sites were identified. The molecular dynamics studies and principal component analysis indicated the stability of the systems and the collective motions, respectively. Unfolding studies suggest that FgGST1 is a highly cooperative molecule because, during GdnHCl-induced denaturation, a simultaneous unfolding of the protein without stabilization of any partially folded intermediate is observed. The protein is stabilized with a conformational free energy of about 10±0.3 kcal mol−1.

cDNA clones encoding a 28-kDa subunit glutathione S-transferase (GST) from Schistosoma mansoni (Sm28GST) and a 26-kDa subunit GST from Schistosoma japonicum (Sj26GST) have been expressed in bacterial systems. The recombinant proteins were purified to homogeneity by batch-wash glutathione-agarose affinity chromatography and their biochemical properties investigated. Gel filtration chromatography indicated that both recombinant GSTs are homodimeric proteins. Resolution of Sm28GST and Sj26GST by chromatofocusing in the ranges pH 9-6 and pH 7-4 gave pI estimates of 7.4 and 5.0, respectively. Kinetic analyses suggested that both Sm28GST and Sj26GST operate via a sequential bisubstrate catalytic mechanism. Sm28GST and Sj26GST displayed a mosaic of mammalian Alpha-, Mu- and Pi-type substrate specificities and inhibitor sensitivities. However, multivariate analysis suggests that Sm28GST has an overall catalytic homology with mammalian Mu class GSTs, whilst the enzymatic properties of Sj26GST appear to constitute a hybridisation of Mu and Alpha class features. Both recombinant GSTs interact with a range of hydrophobic ligands including haematin and related compounds, bile acids and several anthelmintics. Sm28GST and Sj26GST possess relatively limited selenium-independent glutathione peroxidase activities, but are able to catalyse the glutathione conjugation of members of the trans,trans-alka-2,4-dienal, trans-alk-2-enal and 4-hydroxyalk-2-enal series of reactive carbonyls (known secondary products of lipid peroxidation).

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