

Thermodynamic analysis of ligand binding to the G-site of rGST M1-1

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The thermodynamics of binding of both the substrate glutathione (GSH) and its structural analogue glutathione sulphonate (GSO_3^-), was investigated by isothermal titration calorimetry. Calorimetric measurements indicated that the binding of both ligands was enthalpically favourable and entropically unfavourable. The changes in enthalpy, entropy and Gibbs free energy were more negative for GSH binding than for GSO_3^- binding. The effects of temperature and protonation on the energetics of the interaction between rGST M1-1 and the two ligands were investigated. Calorimetric titrations into several buffers with different ionisation enthalpies indicate a release of protons upon GSH binding, whereas GSO_3^- binding shows no coupling to a protonation/deprotonation process at pH 6.5. At 25°C both interactions are characterised by a monophasic binding isotherm with K_d values of 38.1 μM for GSH binding and 2.8 μM for GSO_3^- binding. Enthalpic and entropic contributions for both interactions exhibited temperature dependence over the temperature range studied (10-25°C). Due to enthalpy-entropy compensation, Gibbs free energy remained constant with increasing temperature. The temperature dependence of the binding enthalpy yielded heat capacity changes of -1.42 kJ/mol/K and -1.64 kJ/mol/K for GSH and GSO_3^- binding, respectively. The more negative heat capacity change suggests a more hydrophobic interaction between rGST M1-1 and GSO_3^- than between rGST M1-1 and GSH.