The role of a mixed charge cluster in the dimerisation of class Mu glutathione transferase

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It has been postulated that the rGSTM1-1 mixed charge cluster at the dimer interface is primarily responsible for subunit association. Analyses of individual rGSTM1-1 subunits demonstrated the absence of any charged clusters. This suggests that the mixed charge cluster forms only upon dimerisation and reinforces the probability that quaternary structure stabilisation is a major role of the mixed charge cluster. Using site-directed mutagenesis, two amino acid substitutions were introduced at the subunit interface. Firstly, R81A which disrupted the mixed charge cluster and secondly, F56S removing the hydrophobic lock-and-key motif. SEC-HPLC of the F56S/R81A enzyme identified that these substitutions resulted in a catalytically inactive yet stable monomeric protein variant. Structural investigations of the monomer by far-UV and near-UV circular dichroism and tryptophan fluorescence revealed a similar secondary structural content and less buried micro-environments surrounding the tryptophans when compared to rGSTM1-1. The blue shift observed as a result of ANS binding depicts a large increase in the accessible hydrophobic surface area of the F56S/R81A variant. A significant decrease in the conformational stability together with a large increase in the solvent exposed surface area of the monomer was determined by the thermodynamic parameters generated from urea-induced equilibrium unfolding. In conclusion, the mixed charge cluster at the dimer interface plays an important role in the quaternary stability of rGSTM1-1 and is essential for monomeric association which in turn is a prerequisite for enzymatic activity.