ABSTRACT

The three dimensional native structure of multi domain proteins is only achieved when the adjacent domains recognise each other through the domain-domain interface. The domain-domain interface of the Glutathione *S*-transferase (GST) family has been studied extensively; however, no studies have been conducted on the role of the linker regions in the domain-domain interactions. Glutaredoxin 2 (Grx2) protein, from the GST family was chosen as model to investigate the possible role of linkers in protein stability by mutational analysis. Bioinformatics data revealed a conserved residue within the linker region (Leu78 in Grx2). A Grx2 mutant was created by replacing the conserved residue (Leu78) within the linker region with an alanine. This mutation (Leu to Ala) was performed in order to assess the role of the conserved residue leucine; whilst maintaining Grx2 function. A previous Grx2 mutant (Grx2 Y58W) was utilised because it incorporates tryptophan into domain 1; therefore it was possible to follow tertiary structural changes in this domain. Grx2 Y58W was compared against the mutant created within the linker Grx2 Y58W/L78A. Far-UV CD spectrum indicated that there was an increase of (~30 %) in ellipticity of Grx2 Y58W/L78A protein whereas; tryptophan fluorescence probes indicated no change in tertiary structure. Conformational stability studies showed a decrease of $\Delta\Delta G\ (H_2O)$ = 3.8 kcal.mol^{-1} due to the impact of the Y58W/L78A mutation. The *m*-value which is indicative of the co-operativity between the two domains has decreased slightly by ~0.4 kcal.mol^{-1} M^{-1}. This reduction in the *m*-value suggested the formation of intermediate however; it was not evident when using ANS as a probe. This study indicates that replacing a leucine with an alanine in the linker region causes a reduction in domain co-operativity. Therefore, the linker region in addition to separating the two domains plays a role in interdomain co-operativity.