Proline *cis-trans* Isomerization in the Folding of Glutaredoxin 2

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The role of the conserved *cis*-proline, at the N-terminus of the beta-strand 3, in the folding of the Glutathione transferase family was determined for Glutaredoxin 2. Equilibrium unfolding studies revealed a two-state unfolding transition with an *m*-value of 2.7 kcal\mol\M urea and a $\Delta\ G(H_2O)$ of 12.3 kcal\mol. Kinetic single and double mixing experiments at 20 degrees celsius, were used to further analyse the (un)folding of Grx2. Changes on (un)folding were monitored by intrinsic tryptophan fluorescence. Two unfolding phases were observed for single-mixing kinetic studies. Three phases were observed for the single-mixing refolding kinetic studies of Grx2. At 1 M residual urea the time constants for the fast, intermediate and slow phases of refolding were 61 milliseconds, 0.79 seconds and 180 seconds respectively. Based on this the slow phase of refolding was proposed to involve the isomerization of the Val48-Pro49 *cis*-peptide bond. An increase in the slow phase on increase in unfolding delay time for double-mixing experiments, confirmed that the slow phase involved the isomerization event. Further conformation was that the rate of the slow phase increased 4 fold with the addition of 3 μM hFKBP-12 to the refolding buffer in single-mixing refolding experiments. A parallel folding pathway involving the fast, intermediate and slow phases is proposed for Grx2.