Determining the role of a topologically conserved isoleucine residue in the structure and function of the glutathione transferase family

Fisher, L., Burke, J. and Dirr, H.W.

Protein Structure-Function Research Programme, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg 2050, South Africa

Certain members of the thioredoxin superfamily that possess the thioredoxin fold contain a highly conserved SNAIL/TRAIL amino-acid sequence motif. Within this motif is a highly conserved isoleucine residue. The high level of conservation of this residue suggests a possible role in the structure, stability, folding or catalytic function of those proteins possessing it. In this study human class alpha glutathione transferase (hGST A1-1) was used as a model to investigate the role of Ile71. The isoleucine residue was substituted for a valine, thus truncating the isoleucine side-chain by one methyl group. This was predicted to create a cavity in the hydrophobic core of domain one while maintaining the non-polar hydrophobic character exhibited by the isoleucine. The substitution had no effect on the global structure of hGST A1-1 as shown by fluorescence and far UV circular dichroism spectroscopy. However the mutation produced a 40% decrease in stability of the protein. The catalytic behavior of hGST A1-1 was significantly increased by the mutation as demonstrated by increased enzyme activity, lowered K_m for the substrates glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB), and increased catalytic efficiencies. Overall, the data from this study shows that Ile71 plays an important role in the enzyme activity although structural data shows the residue is not directly involved in the active site of the enzyme.