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Structural characterization and functional studies of putative human glutathione-specific γ-glutamylcyclotransferase 2 (ChaC2 enzyme)

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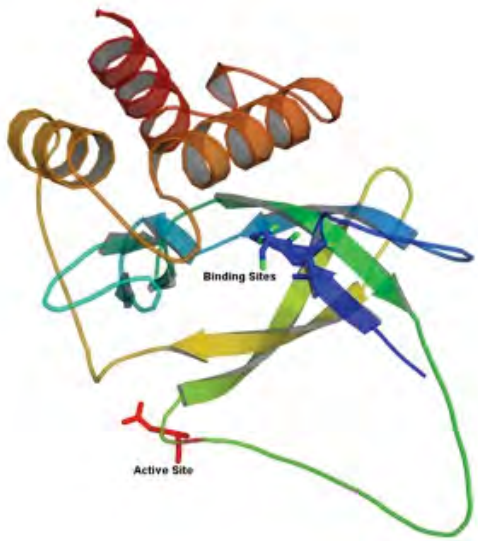
Putative glutathione-specific γ-glutamylcyclotransferase 2 (ChaC2) enzyme degrades glutathione which is involved in various signaling pathways and cellular detoxification. To elucidate its catalytic mechanism, we implemented structural biology approaches for ChaC2. Since SeMet-based phasing methods are generally applicable to proteins with at least one methionine per 100 amino acid residues, ChaC2 was a borderline case. We mutated several leucine and isoleucine residues of ChaC2 to methionine residues to enhance the phasing power for multi-wavelength anomalous diffraction (MAD) method and hence structure determination. From structure analysis and alignment with other γ-glutamylcyclotransferase, we hypothesized that Glu83 of ChaC2 may play an important role in enzyme activity of ChaC2. We crystallized and obtained the structure of ChaC2 E83Q mutant by molecular replacement method. Excitingly, the structure of this mutant differed from that of the wild type suggesting the vital role of this residue. Our results provide an insight on the effect of a mutation on enzyme activity of ChaC2 and its role in cell. Further experiments need to be performed to elucidate the mechanism of how ChaC2 can cleave specifically on glutathione.



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