Title:	Structural and Functional Analysis on ChaC Enzymes
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Keywords:	Biology Enzymes Glutathione ChaC Enzymes
Issue Date:	9-Aug-2016
Publisher:	IISER-M
Abstract:	Glutathione is an abundant non-protein thiol compound present in almost all eukaryotic cells. It is a potent antioxidant and an important redox buffer. Maintenance of GSH homeostasis in the cell is very important and it is accomplished by GSH synthesis, transportation and degradation. Glutathione is degraded by different enzymes: DUG pathway, γ- Glutamyl transpeptidase, ChaC family. ChaC family enzymes are present across phyla. In humans two ChaC enzymes are present: ChaC1 and ChaC2. GCG1 is the homologue of human ChaC2 found in S.cerevisiae. In vitro kinetic studies reveal that ChaC1 is 20 fold more catalytically active than ChaC2. Our current study focuses on finding the amino acid residues that are responsible for the high catalytic activity of ChaC1 using a mutagenesis approach. A total of 13 residues in ChaC2 or GCG1 were mutated to the corresponding ChaC1 residues. All these changes lead to either no change or a further decrease in catalytic activity in ChaC2 or GCG1. Only one residue Phe146 of ChaC2 was when changed to Leu showed an increase in activity. This data needs to be validated by in vitro studies. Another important aspect of GSH is its compartmentalization in various cellular organelles. The role of GSH in these organelles is not yet studied properly. We made an attempt to study the role of GSH in the nucleus. We are using a novel approach of targeting GCG1 (GSH degrading enzyme) to the nucleus for specifically depleting nuclear glutathione and have studied its effects on growth and sensitivity of nuclear DNA to damage. However the nuclear localization of GCG1 still needs to be confirmed.
URI:	http://hdl.handle.net/123456789/586 (http://hdl.handle.net/123456789/586)
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