**γ-Glutamylcysteine Synthetase: Peptide Formation Coupling with ATP Hydrolysis**

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γ-Glutamylcysteine synthetase (GCS), a rate-limiting enzyme in glutathione biosynthesis, plays a central role in glutathione homeostasis and adaptive responses to stress conditions that increase the levels of reactive oxygen species. The catalytic mechanism of GCS involves the initial activation of the γ-carboxyl group of L-Glu by ATP-phosphorylation to form a γ-glutamylphosphate intermediate, followed by the nucleophilic attack of L-Cys to generate a tetrahedral transition state. In order to capture the transient steps of the catalytic mechanisms coupling between peptide formation and ATP hydrolysis, we determined the five crystal structures of GCS in complex with substrates, transition-state analogs and products.

Positional shifts of the phosphate group transferred from ATP and the bound magnesium ions induce conformational changes of two variable arms (residues 105-144 and 240-298) [1]. These arm's movements cause the side chain of Tyr-300 to turn and form a hydrogen bond with cysteine substrate, allowing the amino group of the cysteine to approach the proposed γ-glutamylphosphate intermediate. The changes of binding modes of nucleotide and amino acid substrates and the corresponding protein structural changes are correlated with the sequence of events occurring along the reaction coordinate and suggests the interesting mechanism of coupling between phosphate transfer and peptide formation.

[1] Hibi *et al*., *Proc. Natl. Sci. Acad. USA*, 2004,**101**, 15052.

**Keywords: glutathione, transition-state analog, coupling reaction**