

Observation of Conformation-Specific Intermediates in Auto-proteolytic Activation

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Posttranslational proteolytic processing plays a critical role in diverse biological processes. Glutaryl 7-aminocephalosporanic acid acylase (GCA) undergoes auto-proteolytic reactions, consisting of primary intramolecular and secondary intermolecular self-cleavages. They occur spontaneously to allow maturation of the precursor to a native enzyme, ($\alpha\beta$)₂ heterotetramer and the release of a 9 amino acid pro-segment. We have solved the crystal structures of GCA native and mutants, and were able to model precursor, intermediates and native conformations during autoproteolytic enzyme activation. Distinctly different conformations of the pro-segment are observed, namely, cleavable, non-cleavable, and dissociated forms. The intra and intermolecular autoproteolytic activation mechanism of GCA is described. Mutagenesis and structural data also support the important role of water molecule in GCA hydrolytic activity.