

Detailed mechanism of catalysis by tetrameric L-glutaminase-asparaginase from *Pseudomonas* 7A (PGA)

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L-asparaginases (EC 3.5.1.1) are widely distributed enzymes among both bacterial and eukaryotic organisms. For over 40 years, L-asparaginases have played a critical role in the treatment of juvenile leukemias and lymphomas. Their primary biochemical function is to catalyze the hydrolysis of L-Asn to L-Asp. Most L-asparaginases also catalyze the hydrolysis of L-Gln to L-Glu, and those that exhibit glutaminase activity that is comparable or higher than asparaginase activity are often referred to as glutaminases-asparaginases. Based on extensive structural and functional studies of L-glutaminase-asparaginase from *Pseudomonas* 7A (PGA), we were able to show unequivocally that the reaction catalyzed by this enzyme proceeds through formation of a covalent intermediate and utilizes a common ping-pong catalytic mechanism consisting of two subsequent nucleophilic substitutions, as previously observed for EcAII. Additionally, by confirming that the same mechanism applies to L-Asn and L-Gln, we postulate that it is common for all these structurally related enzymes. Detailed structural studies of PGA and its complexes with substrates should create a foundation for rational development of L-asparaginases with modulated relative activities vs. L-Asn or L-Gln, which may be beneficial toward the development of improved anti-leukemia therapeutics.