The three-dimensional structure of *Escherichia coli* asparagine synthetase B: a short journey from substrate to product

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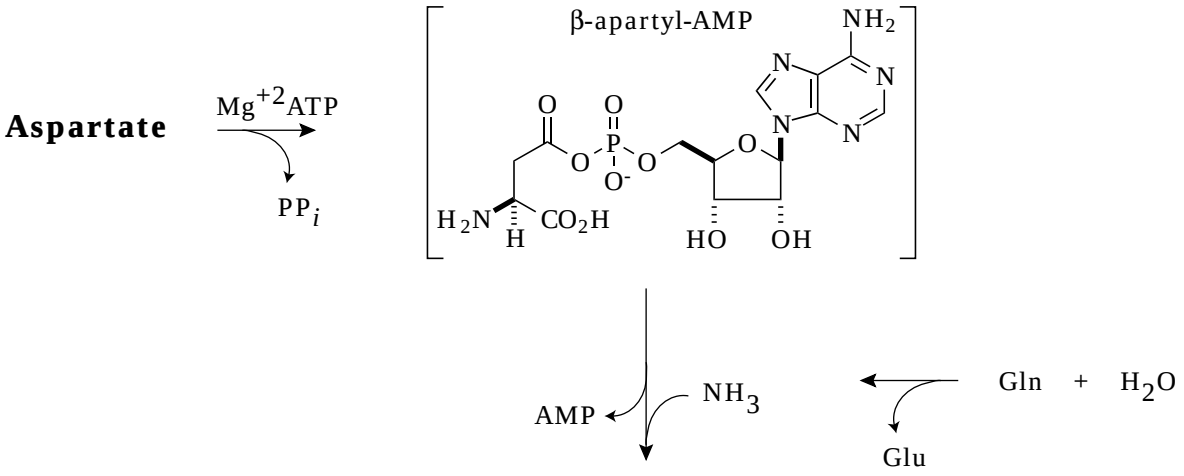
Asparagine synthetase B catalyzes the assembly of asparagine from aspartate, Mg+2ATP, and glutamine. Asparagine synthetase, isolated from both prokaryotes and eukaryotes, catalyzes the ATP-dependent conversion of aspartic acid to asparagine, employing either glutamine or ammonia as the nitrogen source. In *Escherichia coli*, there are two unlinked genes, asnA and asnB, that code for mechanistically distinct enzymes. Asparagine synthetase A is strictly ammonia-dependent whereas the preferred source of nitrogen for asparagine synthetase B is glutamine. According to the presently available experimental data, the overall transformation of aspartic acid to asparagine occurs via three separate reactions as indicated in Scheme 1: the activation of aspartate by the formation of a b-aspartyl-AMP intermediate, the hydrolysis of glutamine to glutamate and ammonia, and the final breakdown of the b-aspartyl-AMP intermediate via a nucleophilic attack by the ammonia.

We describe the three-dimensional structure of the enzyme from *Escherichia coli* determined via a uranium multiwavelength anomalous dispersion experiment and refined to 2.0 Å resolution. Protein employed for this study was that of a site-directed mutant protein, Cys1Ala. Large crystals were grown in the presence of both glutamine and AMP. Each subunit of the dimeric protein folds into two distinct domains. The N-terminal region contains two layers of antiparallel β-sheet with each layer containing six strands. Wedged between these layers of β-sheet is the active site responsible for the hydrolysis of glutamine. Key side chains employed for positioning the glutamine substrate within the binding pocket include Arg 49, Asn 74, Glu 76, and Asp 98. The C-terminal domain, responsible for the binding of both Mg+2ATP and aspartate, is dominated by a five-stranded parallel β-sheet flanked on either side by α-helices. The AMP moiety is anchored to the protein via hydrogen bonds with Og of Ser 346 and the backbone carbonyl and amide groups of Val 272, Leu 232, and Gly 347. As observed for other amidotransferases, the two active sites are connected by a tunnel lined primarily with backbone atoms and hydrophobic and nonpolar amino acid residues. Strikingly, the three-dimensional architecture of the N-terminal domain of asparagine synthetase B is similar to that observed for glutamine phosphoribosylpyrophosphate amidotransferase while the molecular motif of the C-domain is reminiscent to that observed for GMP.



Scheme 1

Scheme 1: The overall transformation of aspartic acid to asparagine.