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Structural and biochemical studies of different forms of bacterial-type L-asparaginases, enzymes used as anti-leukemia drugs

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Anticancer activity of L-asparaginase (ASNase) has been known for well over half a century [1] and an enzyme produced by *Escherichia coli* (named EcAII) has been a major component in treatment of acute childhood lymphoblastic leukemia (ALL) for over 40 years. It was later joined by ErA from *D. dadantii*. These therapies, however, are often associated with serious side effects. It is not yet certain whether the significant glutaminolytic activity, observed for many ASNases, is responsible for some of the side effects, or conversely whether a possible requirement for such an activity is needed for a successful therapeutic outcome.

Based on their structural features, bacterial-type ASNases can be broadly classified into four subgroups, type I and II, each homologous to their respective enzymes in *E. coli*, extremophilic ASNases (i.e. the enzyme from *H. pylori*), and short-chain asparaginases, like ASNase from *R. rubrum* [2,3]. Enzymes from the first three categories contain a polypeptide chain consisting of over 300 amino acids organized in two topological domains, sometimes accompanied by an additional domain, and form homotetramers assembled of two “tight” dimers. These enzymes, often called bacterial-type ASNases, have been also identified in archaea or eukarya. Very poorly characterized short-chain ASNases contain polypeptides forming only a single topological domain corresponding to the N-terminal fragment of more typical ASNases, but the functional form of these enzymes is also a tetramer [3]. Although over 110 structural models of ASNases have been deposited in the Protein Data Bank during the last 30 years [2], an agreement on the enzymatic mechanism of these enzymes has been slow to be reached. Based on results of multipronged studies of type I and II ASNases, we were able to show unequivocally that substrate hydrolysis follows the double displacement (ping-pong) mechanism, with a specific Thr residue being the primary nucleophile [4]. All ASNases share common active sites that are quite distinct compared to their counterparts in other hydrolases. Residues from both protomers forming a tight dimer contribute to the active site pocket. A motif of three residues, Asp-Lys-Thr, reminiscent of the ‘catalytic triad’ in serine proteases, is present in all bacterial-type ASNases. The threonine residue from this motif, however, does not act as the primary nucleophile. That role is played by another threonine side chain, placed on the opposite side of the substrate molecule and not directly accompanied by a general base. Activation of this threonine is provided by a distant motif of two carboxylate groups (a proton sink) that is formed only after binding of a substrate molecule. The process is mediated by a series of well-positioned hydroxyl groups, contributed primarily by water molecules. Also, the ‘oxyanion hole’, a motif observed in all hydrolases and responsible for stabilization of an anionic tetrahedral intermediate, is unique in L-asparaginases, as it incorporates an invariant water molecule.

We have shown that type I ASNase may be active in a dimeric form [5]. Similar determination is currently not possible for type II ASNases as these enzymes exist exclusively as tetramers even at low nanomolar concentrations. The molecular basis of other important properties of these enzymes, such as their substrate specificity, are still being evaluated. Results of structural and mechanistic studies of L-asparaginases are being utilized in efforts to improve the clinical properties of this important anticancer drug.

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