Title:	BsuAP, An Aminopeptidase from Bacillus subtilis: Structural-Biochemical and mutational exploration of function
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Abstract:	We have characterized a Bacillus subtilis derived thermostable aminopeptidase and shown how this enzyme can be used as a novel reagent for determination of the sequence of any peptide through limited proteolysis of the N-terminus. We have found the aminopeptidase to be processive and also nonspecific in respect of the amino acids that it recognizes and acts upon. The characterization of the enzyme reveals that it undergoes a structural transformation upon heating, associated with optimal function at 70 °C. The native quaternary structure of the enzyme is a dodecamer, which we find forms through the tetramerization of four trimers which are initiall formed. Removal of Zn 2+ from an intermediate state formed during assembly (i.e., from a trimer which displays no independent aminopeptidaase activity) slows down the process of dodecamerization, indicating that Zn 2+ is important for assembly. Removal of Zn 2+ from the dodecamer does not appear to be feasible, because the ion is made inaccessible through burial. The trimer-dodecamer equilibrium is shifted towards formation of the dodecamer under many different conditions, and also affected by exposure to high salt. We have mutated a key lysine residue involved in regulating entry of peptides to the active site(s) inside the hollow dodecamer, and examined the resultant effects on catalytic parameters. Our current view is that the dodecamer with four entry points (or pores created at the interface of monomers assembling to make trimers) regulates the discharge of hydrolyzed N-terminal amino acids which are collected inside the hollow dodecameric structure. We have also tried to create trimeric subunit assemblies through multiple mutations of several interface residues involved in trimer–trimer interactions. The result was the creation of a trimer which is aggregation-prone, with a tendency to display self-proteolysis. Refolding of mutants in the presence of a metal chelator stopped the proteolysis and enhanced folding but was unable to stop the tendency of these mutants to aggregate.
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