Biochemical properties of an archaeal signal peptide peptidase from
*Thermococcus kodakaraensis*

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The molecular mechanism of protein secretion in Archaea is far from fully understood, and identification of the individual factors involved in this process, as well as determining their functions, is an attractive subject for research. In this study, we have focused on the signal peptide peptidase (SPP); an enzyme considered to cleave the signal peptide chains of secreted proteins into shorter peptides after they are removed from their precursor proteins by signal peptidases. *Thermococcus kodakaraensis* KOD1 is a hyperthermophilic archaeon isolated from a solfatara on Kodakara Island, Kagoshima, Japan. The strain is an obligate anaerobe, and grows optimally at 85 degrees. *T. kodakaraensis* degrades and utilizes various polysaccharides, and a number of extracellular enzymes have been identified. The complete genome sequence of *T. kodakaraensis* has recently been determined, and predicted to harbor 2,306 genes.

Although significantly smaller in size than the bacterial counterpart, we noticed the presence of a gene encoding a protein of 334 residues (SppAₜₖ) that displayed similarity to the bacterial SPP from *Escherichia coli* (618 residues). SppAₜₖ harbored a single predicted transmembrane domain near its N-terminus. In order to clarify the function of this protein in *T. kodakaraensis*, a detailed biochemical characterization of SppAₜₖ was performed. We examined a 54 residue N-terminal truncated protein (deltaN54SppAₜₖ), in which the N-terminal transmembrane domain and a region susceptible to autoproteolysis was removed. deltaN54SppAₜₖ exhibited peptidase activity towards fluorogenic peptide substrates and was found to be highly thermostable. Moreover, the enzyme displayed a remarkable stability and preference for alkaline pH, with optimal activity detected at pH 10. We also examined the protease activity of deltaN54SppAₜₖ, and detected activity towards particular protein substrates at 60 degrees, pH10. Degradation of bovine serum albumin and ovalbumin was not observed, while casein, hemoglobin, and lysozyme were degraded to various extents. The substrate specificity of the enzyme was examined in detail with a FRETS peptide library. By analyzing the cleavage products with LC-MS, deltaN54SppAₜₖ was found to efficiently cleave peptides with a relatively small side chain at the P1 position and a hydrophobic or aromatic residue at the P3 position. The positively charged Arg residue was preferred at the P4 position, while substrates with negatively charged residues at the P2, P3, or P4 positions were not cleaved. In the predicted signal sequences from the *T. kodakaraensis* genome data, we observed several sites in each signal sequence that matched the cleavage specificity of SppAₜₖ. Our results support the assumption that SppAₜₖ is responsible for the initial breakdown of free signal peptides, and functions as a signal peptide peptidase in this archaeon. We are at present determining the essential or important residues for the activity of SppAₜₖ through site-directed mutagenesis analysis.