The protease is a metalloprotease most active in 2 mM CaCl₂ and 0.1 mM ZnCl₂. Digestion time can be very rapid, even less than 1 hr. A novel Arg/Lys specific N-terminal peptidase is presented. Detection sensitivity in LOD MRM experiments is increased ~4.5x (median) relative either enzymatically or, with extra steps, by chemical modification. To produce MS/MS spectra. This is achieved by controlling where charge is located on the peptide specificity. The enzyme can be used as a direct substitute for trypsin, generates predominantly N-terminal ions, charge must be placed at or near the N-termini of peptides. This can be most elegantly achieved using a proteolytic enzyme with N-terminus cleavage specificity at basic amino acids. This was achieved partly with Lyt-3, a metalloprotease with N-terminal cleavage specificity only (1-3). Here, we present a novel thermostable protease with equal N-terminal arginine and lysine specificity. The enzyme can be used as a direct substitute for trypsin, generates thermostability, and multiple buffers, pH of 5-12, detergents and salts. It withstands repeated freeze-thaw cycles.

Control of activity

The enzyme exhibited a temperature optimum of 65 °C and a broad pH specificity between 5 – 12. The protease was most active in low ionic strength and SDS compatible buffers, especially ssDNA. Enzymatic activity was most efficient in the presence of calcium and zinc. In contrast to trypsin, MS/MS protease or selectively cleavable detergents did not improve digestion nor reduction of identified peptides or proteins. Specificity for trypsin-like cleavage at arginine and basic residues was ~50%, with the remaining ~50% to other amino acids. More than 50% of identified peptides and proteins were similar to trypsin, yet generated a nearly 20x shorter digestion time in a similar incubation time. In complex mixtures, a shift to lower pH decreased enzyme activity and, although specificity is not significantly decreased, longer incubation times result in fewer detectable peptides.

**Conclusions**

The novel Arg/Lys specific N-terminal protease offers a significant improvement in the field of proteomics. It provides more interpretable spectra, containing proline or those with histidine at or near the C-terminus. In complex mixtures, a shift to lower pH decreases enzyme activity and, although specificity is not significantly decreased, longer incubation times result in fewer detectable peptides. This is likely due to the enzyme's ability to cleave with extended exposure at elevated temperatures.

**Materials and methods**

A novel Arg/Lys specific N-terminal protease was presented. Digestion efficiency was measured and conditions were optimized for temperature, incubation time, and concentration of basic amino acids. Digestion efficiency was assessed with trypsin-derived peptides from proteinase K digests and subsequent LC-MS/MS analysis. Digestion efficiency was assessed for the protease and further confirmed by trypsin digestion of tryptic peptides from a tryptic digest of rat liver and subsequent LC-MS/MS analysis. The protease was digested in 0.1% TEAA and a broad pH specificity between 5 – 12. The protease was most active with ssDNA and, although specificity is not significantly decreased, longer incubation times result in fewer detectable peptides. This is likely due to the enzyme's ability to cleave with extended exposure at elevated temperatures.

**III. Introduction**

Directed single proteolytic events can generate specific signal transduction events and simplify MS/MS spectra. This is achieved by controlling where charge is located on the peptide other enzymes can result in the need for chemical modification. To produce MS/MS spectra. This is achieved by controlling where charge is located on the peptide specificity. The enzyme can be used as a direct substitute for trypsin, generates predominantly N-terminal ions, charge must be placed at or near the N-termini of peptides. This can be most elegantly achieved using a proteolytic enzyme with N-terminus cleavage specificity at basic amino acids. This was achieved partly with Lyt-3, a metalloprotease with N-terminal cleavage specificity only (1-3). Here, we present a novel thermostable protease with equal N-terminal arginine and lysine specificity. The enzyme can be used as a direct substitute for trypsin, generates thermostability, and multiple buffers, pH of 5-12, detergents and salts. It withstands repeated freeze-thaw cycles.

Control of activity

The enzyme exhibited a temperature optimum of 65 °C and a broad pH specificity between 5 – 12. The protease was most active in low ionic strength and SDS compatible buffers, especially ssDNA. Enzymatic activity was most efficient in the presence of calcium and zinc. In contrast to trypsin, MS/MS protease or selectively cleavable detergents did not improve digestion nor reduction of identified peptides or proteins. Specificity for trypsin-like cleavage at arginine and basic residues was ~50%, with the remaining ~50% to other amino acids. More than 50% of identified peptides and proteins were similar to trypsin, yet generated a nearly 20x shorter digestion time in a similar incubation time. In complex mixtures, a shift to lower pH decreases enzyme activity and, although specificity is not significantly decreased, longer incubation times result in fewer detectable peptides. This is likely due to the enzyme's ability to cleave with extended exposure at elevated temperatures.