

**ION TRAP MASS SPECTROMETRIC MONITORING OF PREFERRED CLEAVAGE SITES  
IN PEPTIDE ENZYMATIC HYDROLYSIS**

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A range of bacteria, including human pathogens, rely on amino acid fermentation for energy. The bacteria may take up free amino acids or peptides present in the environment, however, they often generate peptides of variable length by digesting host proteins using proteases secreted into the environment. The bacterial peptide transport systems and their requirements in terms of peptide characteristics, such as, sequence, charge or hydrophobicity are largely unknown for many pathogens. However, a first insight can be achieved by determination of the specificity of the proteolytic enzymes secreted by the bacteria. This can be determined by incubation of the bacteria or purified extracellular enzymes with a known protein and measurement of the resulting peptides. Ion trap electrospray mass spectrometry lends itself perfectly to this type of measurement. We report the use of the ion trap mass spectrometer to monitor hydrolysis of the salivary peptide histatin by the major extracellular proteinase complex of the oral pathogen *Porphyromonas gingivalis* as well as histatin hydrolysis by trypsin as a control. Histatin was incubated in a syringe with the enzyme complex or trypsin and the contents of the syringe were slowly electrosprayed into the ion trap MS. The generated ions were continuously measured and the resulting spectra showed the progressive hydrolysis of histatin.

The sequence of histatin has several potential tryptic cleavage sites as shown below.

1            6            12            17            22 24  
DSHAKRHHGYKRFKHEKHSHRGY

Trypsin cleaves first at Arg<sup>12</sup>, while the *P. gingivalis* enzyme complex cleaves first at Arg<sup>22</sup> followed by cleavage at Arg<sup>6</sup> and Lys<sup>17</sup>, and then at Arg<sup>12</sup>. This shows that although trypsin and the enzyme complex have identical specificity for Arg and Lys, the actual site preference for hydrolysis is influenced by other factors, such as, adjacent amino acids and secondary structure.