

## Enzyme, Mr, pI and Specificity

Enzyme	Molecular Weight	pI
Chymotrypsin, Alpha	25,000	9.1
Trypsin	23,800	10.5
Elastase	25,900	8.5
Papain	23,400 (Theoretical), 23,000 (Dreuth et al. 1968)	6.8(Theoretical), 8.75 (Experimental)
Subtilisin	27,287	9.4
Endoproteinase Glu-C	29,020 (Sigma), 27,000 (Drapeau 1978)	N/A
Proteinase K	28,900	8.9 (Sigma)
Clostripain	50,000 (Mitchell and Harrington 1968), Two chains of 45,000 and 12,500 (Gilles et al. 1978)	4.8-4.9
Pepsin	34,600	1.0 (Bovey and Yanari 1960)
Thermolysin	34,600	4.45
Bromelain	22,500 (Wharton 1974)	9.55
Actinase E	20,000	N/A

- Chymotrypsin preferentially cleaves peptide amide bonds where the carboxyl side of the amide bond (the P1 position) is a tyrosine, tryptophan, or phenylalanine. These amino acids contain an aromatic ring in their side chain that fits into a 'hydrophobic pocket' (the S1 position) of the enzyme. The hydrophobic and shape complementarity between the peptide substrate P1 side chain and the enzyme S1 binding cavity accounts for the substrate specificity of this enzyme. [2][3] Chymotrypsin also hydrolyzes other amide bonds in peptides at slower rates, particularly those containing leucine at the P1 position.
- Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline.
- Elastase cleaves peptide chains mainly at the carboxy side of small, hydrophobic amino acids such as glycine, alanine, and valine.
- Papain will digest most protein substrates more extensively than the pancreatic proteases. Papain exhibits broad specificity, cleaving peptide bonds of basic amino acids, leucine, or glycine. It also hydrolyzes esters and amides. Papain exhibits a preference for an amino acid bearing a large hydrophobic side chain at the P2 position. It does not accept Val at the P1' position.
- Subtilisin is a member of the Serine S8 Endoproteinase family. It has broad specificity with a preference for a large uncharged residue in the P1 position. It hydrolyzes native and denatured proteins, and is active under alkaline conditions.
- Endoproteinase Glu-C from *Staphylococcus aureus* strain V8 is a serine endoprotease, which hydrolyzes peptide bonds at the carboxyl side of glutamyl and aspartyl residues. The specificity of Glu-C is dependent upon the buffer and pH employed as well as the structure around the potential cleavage site. In ammonium acetate (pH 4.0) or ammonium bicarbonate (pH 7.8) the enzyme preferentially cleaves glutamyl bonds; whereas, in phosphate buffer (pH 7.8) Glu-C will cleave at either site. No cleavage will occur if a proline residue is on the carboxyl side. The enzyme also exhibits esterase activity.
- Proteinase K is a stable serine protease with broad substrate specificity. The predominant site of cleavage is the peptide bond adjacent to the carboxyl group of aliphatic and aromatic amino acids with blocked alpha amino groups.
- Clostripain cleaves proteins on the carboxyl peptide bond of arginine.
- Pepsin is most efficient in cleaving peptide bonds between hydrophobic and preferably aromatic amino acids such as phenylalanine, tryptophan, and tyrosine.
- Thermolysin specifically catalyzes the hydrolysis of peptide bonds containing hydrophobic amino acids.
- Bromelain is a cysteine endopeptidase with broad specificity for cleavage of proteins.
- Actinase E is a serine protease displaying a wide range of substrate specificities, which are believed to be mediated by an active site composed of one Asp, one His, and a Ser residue in the molecule. Actinase E prefers to hydrolyze peptide bonds on the carboxyl side of glutamic or aspartic acid.