Evolution and Cleavage Specificity of Hematopoietic Serine Proteases

Vamsi Boinapally

Several of the major cell types of the immune system store large amounts of serine proteases in their cytoplasmic granules. They have got their name from the serine residue that is responsible for catalytic mechanism. Serine along with histidine and aspartic acid residues are responsible for peptide bond hydrolysis. Many different functions have been described for these serine proteases, like induction of apoptosis in cells infected with intracellular parasites, remodeling of extracellular matrix and triggering of inflammation by recruiting inflammatory cells. These serine proteases are encoded in four different loci, the mast cell chymase locus, the mast cell tryptase locus, the methase locus and T cell tryptase locus. The serine proteases have been labeled according to their cleavage specificity as chymotrypsin-like, trypsin-like and elastase-like.

Detailed analyses of the chymase locus and the T cell tryptase locus have been the focus of this degree project as well as the analysis of the extended cleavage specificity of the macaque chymase. In order to characterize the extended cleavage specificity (other preferred amino acids around cleaved peptide bond) of the macaque chymase, we used the method of substrate phage display. The phages of the library used encode a nine amino acids long substrate region and a six histidine residues long region following this nine amino acid random region to be able to bind the individual phages to a matrix of nickel-chelating beads. Upon addition of the protease, the phages with random peptide sequences susceptible to protease attack will be released from the beads. Amplification of these released phages and reattachment to nickel beads and subsequent protease cleavage several times results in that only phages susceptible to protease will remain. When this situation has been reached the region of interest of 100 individual phages will be PCR amplified and sequenced. These sequences will be translated and aligned to get a consensus cleavage specificity of the protease

My analysis of the extended cleavage specificity of the macaque chymase has shown that this enzyme has very similar, almost identical, cleavage specificity as the human chymase.

My analysis of the evolution of the chymase and granzyme A-K loci have shown that the chymase locus has expanded quite dramatically during the past 150 million years of mammalian evolution, particularly in rodents. In contrast the granzyme A-K locus has suffered relatively few changes during 450 million years of vertebrate evolution.

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Biology Education Centre and the Department of Cell and Molecular Biology, Uppsala University
Supervisor: Lars Hellman