Examination of potential interaction between Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) and Major Histocompatibility Complex I (MHC I)

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MHC I is a transmembrane heterodimeric protein complex, which is expressed in every nucleated cell and presents antigenic peptides (usually 8-10 aminoacids in length) to $CD8^+$ cytotoxic T-cells, thus initiating adaptive immune response. These peptides originate from N-terminally extended peptide precursors which enter the endoplasmic reticulum after degradation of intracellular proteins by the proteasome. ERAP1 crucially participates in the antigen presentation pathway and shaping of immunopeptidome, as it processes the N-terminally extended precursors to either optimal length for MHC I binding or it destroys them. However, the physiological substrate of ERAP 1 has been controversial, since two molecular pathways of trimming have been proposed, without being mutually exclusive: The first mechanism suggests that ERAP1 trims antigenic peptide precursors in solution while the second claims that the enzyme can process the precursors while the latter are partially bound onto MHC I. While the first mechanism has been well established in the literature, certain studies have suggested that the second mechanism is also possible. In this study we aimed to investigate the potential interaction between ERAP1 and MHC I by attempting to assemble a stable trimeric ERAP1-peptide-MHCI complex. For this purpose, we utilized model peptides that carry pseudopeptide phosphinic groups at their N-terminus that present high affinity for the ERAP1 active site. The peptides are covalently linked to the peptide-binding groove of the MHCI alleles B*08:01 or A*02:01 respectively via a disulfide bond with an engineered cysteine residue introduced after site-directed mutagenesis. Efforts to assemble this trimeric complex are described.