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Review

# Antigenic peptide trimming by ER aminopeptidases—Insights from structural studies☆

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ABSTRACT

Generation and destruction of antigenic peptides by ER resident aminopeptidases ERAP1 and ERAP2 have been shown in the last few years to be important for the correct functioning and regulation of the adaptive immune response. These two highly homologous aminopeptidases appear to have evolved complex mechanisms well suited for their biological role in antigen presentation. Furthermore, polymorphic variability in these enzymes appears to affect their function and predispose individuals to disease. This review discusses our current understanding of the molecular mechanisms behind ERAP1/2 function as suggested by several recently determined crystallographic structures of these enzymes.



## 1. Biology of ERAP1/2

The human adaptive immune system identifies diseased and aberrant cells by monitoring the cell-surface presentation of the peptide products of proteolytic digestion of intracellular and endocytosed proteins (Rock and Goldberg, 1999), which are indicative of the overall cellular protein content. Peptides are presented by MHC-I and MHC-II proteins for recognition by receptors on T cells. In general, peptides bound by MHC-I proteins require proteolytic processing before binding. The proteolytic pathway that leads to the generation of most antigenic epitopes starts at the proteasome and ends with a series of trimming events in the endoplasmic reticulum by the ER-resident aminopeptidases ERAP1 and/or ERAP2. Peptides longer than 8–10 residues (the optimum length for binding to most MHC-I allelic variants) can be N-terminally processed in the ER by ERAP1 and/or ERAP2 to generate the correct-length mature antigenic peptide, however, this processing can also destroy epitopes by trimming to lengths too small to bind onto MHC class I molecules. The importance of ERAP1 and ERAP2 for the generation of several key antigenic epitopes that relate to human disease has been thoroughly demonstrated both *in vitro* and *in vivo* [reviewed in Evnouchidou et al. (2009), Haroon and Inman (2010) and Weimershaus et al. (2012)]. Furthermore, the capacity of these two enzymes to destroy epitopes has also been shown to be a fundamental part of their biological role (York et al., 2002). This dual "generate or destroy" role gives these two enzymes the capability to control antigen generation by selecting which epitopes will be efficiently produced and loaded onto MHC class I molecules. As a result ERAP1/2 activity can influence the antigenic peptide repertoire and the resulting immunodominance hierarchy (Blanchard et al., 2008; Hammer et al., 2007; York et al., 2006). These regulatory properties of ERAP1 and ERAP2 have elevated interest in the function of these molecules among immunologists but remain poorly understood at both functional and mechanistic levels.

ERAP1 and ERAP2 are both zinc metalloaminopeptidases that belong to the M1 protease family (Rawlings et al., 2012) characterized by GAMEN and HExxHx$_{18}$E motifs. They are highly homologous (~50% sequence identity) and along with the homologous IRAP (~50% sequence identity) form a gene cluster on chromosome 5 in humans (in mouse, ERAP1 is on chromosome 13, IRAP is on

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chromosome 17, and ERAP2 is absent). All three recently have been classified to the oxytocinase subfamily of M1 aminopeptidases (Tsujimoto and Hattori, 2005). ERAP1 was the first to be associated with a role in antigen processing and as a result this enzyme has been more thoroughly characterized. ERAP1 has molecular and enzymatic properties that are well suited to its specific biological role. Many antigenic peptide precursors that enter the ER are too long to bind to MHC-I proteins, having 1–6 or possibly more amino acids that require trimming (Cascio et al., 2001). In general, aminopeptidases show reduced activity for substrates of that length (10–15 amino acids) and display higher activity for shorter substrates, a property that would promote epitope destruction unless a specific protection mechanism was present. Thus, an aminopeptidase with a role in epitope generation would need an unusual length preference. Furthermore, antigenic peptide precursors vary tremendously in terms of peptide sequence and as a result any aminopeptidase activity (or activities) suitable for trimming them would have to be able to deal with a very large number of different peptide sequences. Biochemical analysis showed that indeed ERAP1 was able to trim larger peptides faster than smaller ones, and that residues throughout the whole length of the enzyme could affect processing rates (Chang et al., 2005; Evnouchidou et al., 2008). These properties suggest that ERAP1 has evolved to fill in this specific biological role and imply that the enzyme could impose a bias on the proteolytic fate of possible epitopes depending on their sequences (Georgiadou et al., 2010). In fact, MHC-I proteins from an ERAP1-deficient mouse carry a spectrum of peptides substantially different than in ERAP1-sufficient animals (Blanchard et al., 2010; Hammer et al., 2007). Less is known about the preferences of ERAP2, in part because of its absence in mouse and consequent lack of a gene-deficient model organism. Initial reports suggest that ERAP2 may not share the length preferences of ERAP1 (Chang et al., 2005). However, ERAP2 has been shown to be important for the trimming of precursor sequences that ERAP1 trims inefficiently, possibly by forming ERAP1/2 functional heterodimers (Saveanu et al., 2005). The third aminopeptidase involved in antigen processing, IRAP, has been shown to generate antigenic epitopes on a discreet pathway of cross-presentation independently of ERAP1 and ERAP2, and to share at least the basic functionality of ERAP1 for efficiently trimming larger precursor sequences (Georgiadou et al., 2010; Saveanu et al., 2009).

## 2. Structures of ERAP1 and ERAP2

During the last two years several three-dimensional structures of ERAP1 and ERAP2 have been solved, a development that has greatly contributed to our understanding of both the mechanism of peptide trimming and substrate selection preferences for these two enzymes (Ascher et al., 2012; Birtley et al., 2012; Evnouchidou et al., 2012; Gandhi et al., 2011; Kochan et al., 2011; Nguyen et al., 2011). Both enzymes show an overall similar domain topology, having four structural domains folding over a concave structure (Fig. 1). Domain II (green in Fig. 1) is the catalytic domain that contains the zinc atom and catalytic motifs. Domain I (cyan) caps off the active site. Domain III (orange) is a small sandwich domain between domains II and IV that acts like a hinge facilitating postulated conformational changes between domains II and IV (see below). Finally, domain IV (magenta) has a concave structure comprised by several helical armadillo-type repeats.

ERAP1 has been shown to adopt at least two distinct conformational states inside the crystal that differ in the orientation of domain IV relative to domains II and I. In one conformation (PDB codes 3MDJ and 3QNF), henceforth termed “open”, domain IV is oriented away from domain II leading to the formation of a large, shallow and solvent exposed cavity starting from the base of the domain I and II interface, extending through the base of the interface of domains III and IV, and ending in the concave section of domain IV (Kochan et al., 2011; Nguyen et al., 2011) (Fig. 2A). In another conformation (PDB code 2YDO), henceforth termed “closed”, the edge of domain IV is juxtaposed onto the edge of domain I, also making interactions with domain II (Fig. 2B). This topology completely excludes the enzyme’s catalytic site from the solvent, but also creates a very large internal cavity, lined by residues of domain II and IV. ERAP2 follows a similar domain topology but has only been crystallized in a configuration similar to the “closed” ERAP1 structure (PDB codes 3SE6 and 4E36) (Birtley et al., 2012; Evnouchidou et al., 2012).

The identification of such a large cavity adjacent to the enzyme’s catalytic center has led researchers to hypothesize that it constitutes a binding site for elongated peptides (Birtley et al., 2012; Gandhi et al., 2011; Kochan et al., 2011; Nguyen et al., 2011). Indeed, the size of the cavity is sufficient to accommodate even the largest of ERAP1’s substrates (16 amino acid long). A series of shallow pockets could provide atomic interactions that drive substrate binding and at the same time display sufficiently broad specificity to allow processing of the large pool of sequences of peptides that ERAP1 is likely to encounter inside the ER. The overall electrostatic potential of this cavity is negative, something that is consistent with the experimental observation that ERAP1 often prefers peptide substrates with positively charged amino acids at several positions in the sequence (Evnouchidou et al., 2008). Comparison between the structural features of this internal cavity for ERAP1 and ERAP2 has suggested that sufficient differences exist between the two enzymes to support different selectivity for substrates (Birtley et al., 2012). However, the absence of crystal structures for complexes of ERAP1/2 with substrates or inhibitors longer than the dipeptide analog bestatin, makes it difficult to unequivocally identify specificity pockets and compare substrate preferences.

Recently, the crystal structure of the isolated C-terminal domain of ERAP1 has been solved, showing a concave configuration highly similar to the one found in the full-length ERAP1 structures (Gandhi et al., 2011). In this structure, a crystallographic dimer is formed by interactions between the C-terminal poly-histidine tag of one monomer and the interior surface of the other. The nature of the interactions as well as the presence of histidine residues in several naturally occurring epitopes prompted the researchers to propose that this interaction mimics the natural binding of a peptide substrate by the enzyme (Gandhi et al., 2011). Indeed, several of the residues implicated have been previously proposed to constitute the C-terminus binding pocket based on the analysis of a full-length ERAP1 crystal structure (Kochan et al., 2011). The recently solved crystal structure of ERAP2, however, revealed that the pocket suggested to be responsible for C-terminus binding in ERAP1 is not conserved in ERAP2, although it should be noted that ERAP2 probably has different length and C-terminal sequence preferences from ERAP1 (Birtley et al., 2012; Chang et al., 2005).

## 3. Conformational changes

The observation of two distinct ERAP1 crystal structures (“open” and “closed”) suggested that the enzyme could undergo a significant conformational change during its catalytic cycle. This idea was further supported by the observation that in the closed structure, the catalytic site and S1 specificity pocket were more organized but lack direct access to the solvent that would be necessary to allow both substrate binding and product release (Kochan et al., 2011; Nguyen et al., 2011). Furthermore, a key active site residue universally conserved at this position in M1 family aminopeptidases, Tyr438, is differently oriented between the two ERAP1 conformations. Mechanistic studies of other M1 family