

**GRADUATE SCHOOL OF BIOMEDICAL SCIENCES**

**BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY**

**Ph.D. THESIS DEFENSE**

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The Relationship Between Inhibition, Conformation, and Catalysis of the Aminopeptidase ERAP1

ERAP1 is an aminopeptidase that is a component of antigen processing. To distinguish the role of ERAP1 from homologs ERAP2 and IRAP, I identified three specific ERAP1 inhibitors via a high-throughput screen. These compounds inhibit hydrolysis of a decamer peptide, and some inhibit ERAP1 in a cellular assay. These inhibitors enable dissection of ERAP1 mechanism. ERAP1 has been crystallized in two conformations: open and closed. I collected SAXS data on ERAP1 in the presence of various inhibitors. ERAP1 adopts an open conformation in solution, but some inhibitors stabilize the closed form. Compound **3**docks to a distal pocket 28Å from the active site zinc, while DG013 and DG014 bind to the active site. This distal pocket is an allosteric activation site, and allostery is mediated by stabilizing the closed state. I also identified an intermediate step in substrate binding where helix 4a becomes ordered while ERAP1 maintains an open conformation. Helix 4a then rotates and engages substrate when ERAP1 closes. The nonsynonymous SNP rs30187 at position 528 (Lys/Arg) subtly alters ERAP1 activity *in vitro*and correlates with disease incidence. Position 528 forms a conformation-dependent electrostatic interaction with Glu913 in the closed structure. The energetic contribution of this interaction is stronger for Lys528 than Arg528. Inhibitors that induce closing are more potent for Lys528 than Arg528. I propose a model where either helix 4a stabilization or allosteric site occupancy shift the conformational equilibrium towards a closed state, while substitution at position 528 alters the opening rate.

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