Polymorphic positions 349 and 725 of the autoimmunity protective allotype 10 of ER aminopeptidase 1 are key in determining its unique enzymatic properties

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ER aminopeptidase 1 (ERAP1) is a polymorphic intracellular aminopeptidase with key roles in antigen presentation and adaptive immune responses. ERAP1 allotype 10 is highly protective towards developing some forms of autoimmunity and displays unusual functional properties, including very low activity versus some substrates [1]. To understand the molecular mechanisms that underlie the biology of allotype 10 we studied its enzymatic and biophysical properties focusing on its unique polymorphisms V349M and Q725R. Compared to ancestral allotype 1, allotype 10 is much less effective in trimming small substrates but presents allosteric kinetics that ameliorate activity differences at high substrate concentrations. Furthermore, it is inhibited by a transition-state analogue via a non-competitive mechanism and is much less responsive to an allosteric small-molecule modulator. It also presents opposite enthalpy, entropy and heat capacity of activation compared to allotype 1 and its catalytic rate is highly dependent on viscosity. Polymorphisms V349M and Q725R significantly contribute to the lower enzymatic activity of allotype 10 for small substrates, especially at high substrate concentrations, influence the cooperation between the regulatory and active sites and regulate viscosity dependence, likely by limiting product release. Overall, our results suggest that allotype 10 is not just an inactive variant of ERAP1 but rather carries distinct enzymatic properties that largely stem from changes at positions 349 and 725. These changes affect kinetic and thermodynamic parameters that likely control rate-limiting steps in the catalytic cycle, resulting in an enzyme optimized for sparing small substrates and contributing to the homeostasis of antigenic epitopes in the ER.

[1] J.P. Hutchinson, I. Temponeras, J. Kuiper, A. Cortes, J. Korczynska, S. Kitchen, E. Stratikos, J. Biol. Chem. 296 (2021) 100443.