Different Effects of the Same Residue Substitution in the Major Cathepsin Ls of the Liver Fluke Fasciola hepatica

Corvo, I.1, O’Donoghue, A. J.2, Pastro, L.1, Pi-Denis, N.1, Eroy-Reveles, A.3, Roche, L.1, McKerrow, J.H.4, Dalton, J.P.5, Craik, C.S.2, Caffrey, C.R.4 & Tort, J.F1

1Departamento de Genética, Facultad de Medicina, 2Department of Pharmaceutical Chemistry, Pharmacology, and Biochemistry and Biophysics, University of California San Francisco (UCSF), California, USA, 3Department of Chemistry and Biochemistry, San Francisco State University, San Francisco, California, USA, 4Center for Discovery and Innovation in Parasitic Diseases, Department of Pathology, UCSF, California, USA; 5Institute of Parasitology, McGill University, Quebec, Canada.

INTRODUCTION: The flatworm Fasciola hepatica is responsible for fasciolosis, one of the most common parasitic diseases of livestock worldwide, with increased incidence of human cases. A family of secreted cathepsin L proteases with differential activities is essential for host colonization. While the blood feeding adult secretes predominantly FheCL1 (an enzyme with a strong preference for Leu occupying the S2 pocket of the active site), the infective stage produces FheCL3 a unique enzyme with collagenolytic activity that favours Pro at P2. We compared substrate specificity of these two enzymes by different methods and made substitutions along S2 and S3 pockets to study their impact in enzyme selectivity.

MATERIAL AND METHODS: Using a novel multiplex substrate profiling and mass spectrometry methodology we compared the preferences of FheCL1 and FheCL3 along the complete active site cleft. We generated variants of the enzymes by directed mutagenesis at S2 and S3 sites and evaluated their substrate specificity using positional scanning synthetic combinatorial libraries and short synthetic peptides. RESULTS AND DISCUSSION: We found differential activity of FheCL1 and FheCL3 sharing only 50% of the cleavage sites, supporting the idea of functional specialization. Besides the rare P2 Pro preference, FheCL3 showed a restricted specificity at the S3 pocket, accommodating preferentially Gly. Both P2 Pro and P3 Gly preferences were ablated when Trp67 of FheCL3 was replaced by Leu rendering the enzyme incapable of digesting collagen. In contrast, the inverse Leu67Trp substitution in FheCL1 only slightly reduced its Leu preference in P2, though increasing accommodation of Gly at S3. CONCLUSIONS: Our data reveal the significance of S2 and S3 interactions in substrate binding emphasizing the role of Trp67 in modulating substrate recognition at both sites. We demonstrate that a single position on the active site, residue 67, is essential to maintain this collagenolytic activity critical for parasite invasion.

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