pH-dependent specificity of papain-like cysteine proteases is determined by S1 binding pocket

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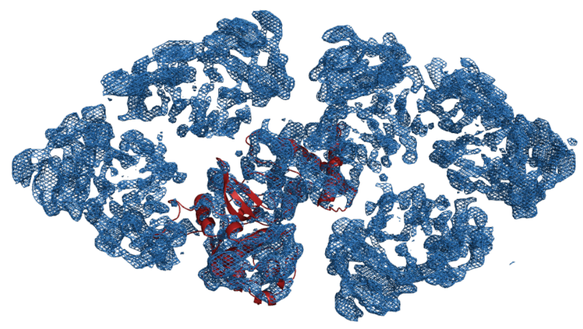
Introduction

Papain-like cysteine proteases (PLCPs) are widely expressed enzymes, the main function of which is low-specific protein turnover in the acidic conditions of lysosomes. Additionally, these proteases provide specific functions in other compartments such as cytosol, nucleus, and extracellular space. The specificity of each protease to its substrates mainly depends on the patterns of the amino acids in the binding cleft.

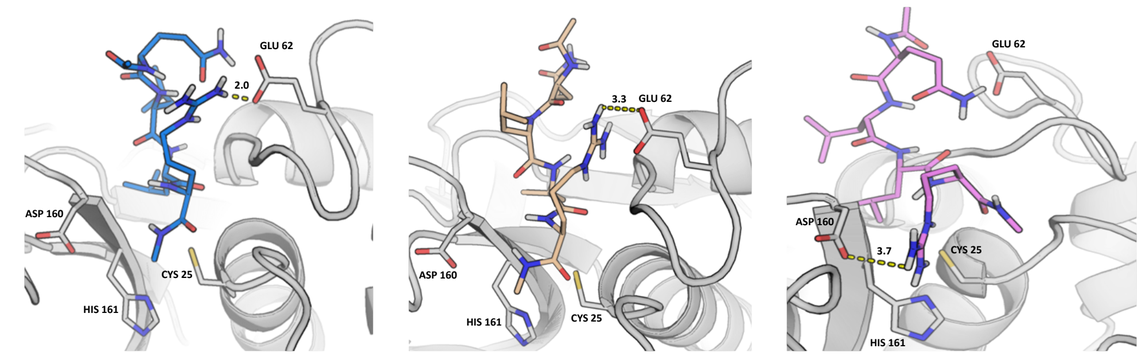
In this study, we examined structural aspects ensuring pH-dependent substrate specificity of PLCPs.

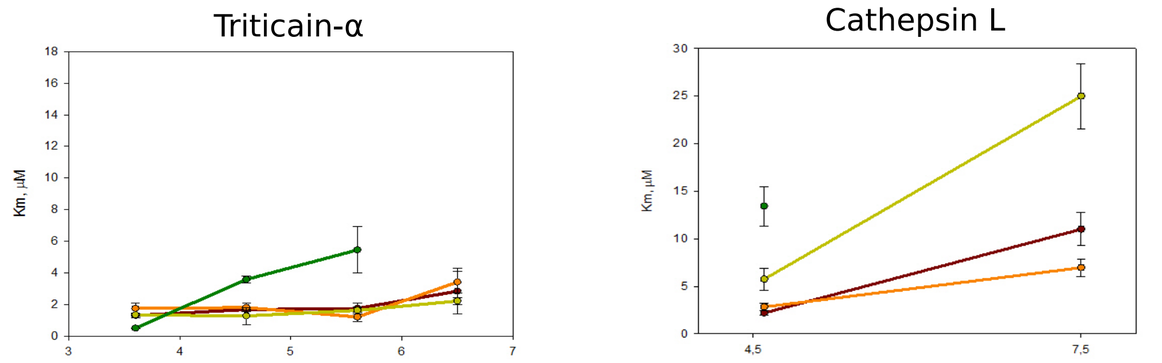
ID	Name	Protease_upper	ID	Name	Protease_lower
QLLRVGCV	ADM2_HUMAN	85,94	QLLRVGCV	ADM2_HUMAN	134,35
VIEFRVMV	detected_by_mass-spec	10,11	VIEFRVMV	detected_by_mass-spec	9,3
NIILSLIM	detected_by_mass-spec	4,67	NIILSLIM	detected_by_mass-spec	9,16
CTDRMTVV	HYCE_ECOLI	16,28	CTDRMTVV	HYCE_ECOLI	8,57
FITDELFSIEVRIAP	random_sequence	4,15	FITDELFSIEVRIAP	random_sequence	7,4
ELNDSTNYIAPKMMP	random_sequence	4,09	ELNDSTNYIAPKMMP	random_sequence	6,74
RNKRAVQG	T13B_HUMAN	7,16	RNKRAVQG	T13B_HUMAN	6,69
GHDQETTT	APM1_HUMAN	5,77	GHDQETTT	APM1_HUMAN	5,69
FPQTAIGV	CAL0_HUMAN	4,48	FPQTAIGV	CAL0_HUMAN	5,03

The PLCP Triticain-α from *Triticum aestivum* screening of protease activity using Protease Profiling on Peptide Microarrays revealed potential substrates of the protease. QLLR sequence is preferable so QLLR and similar substrates, QLLK, QLLQ, and QLLD, were selected for further study of the enzyme specificity.

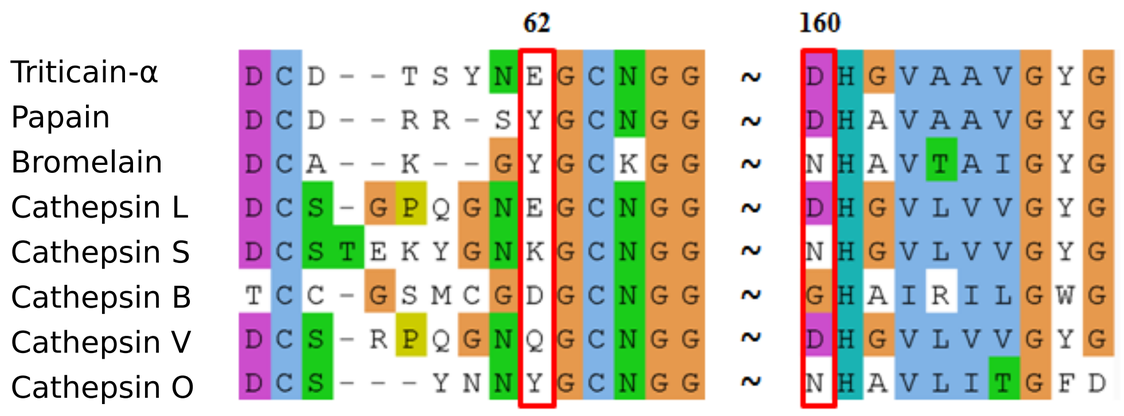


The alanine mutant of Triticain-α was screened against 384 crystallization conditions and found to crystallize in a range of conditions. The structure was obtained at 3.7 Å resolution using structures of other PLCPs: vignain (1S4V) and caricain (1PCI), as molecular replacement models and then it was refined against the electronic density using Rosetta tools.

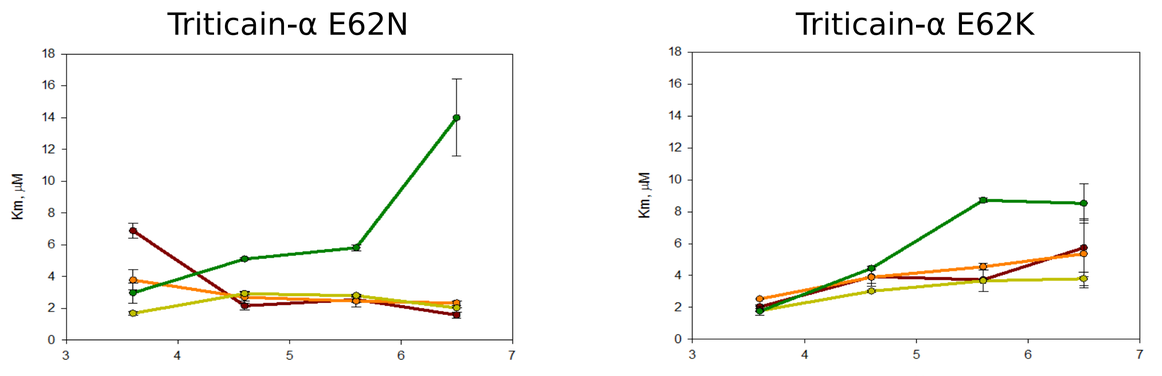


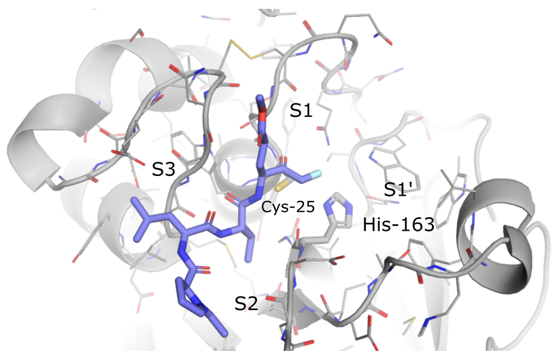


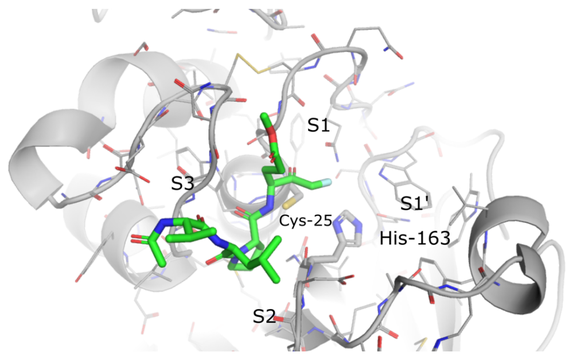
Molecular docking identified in the S1-binding pocket of Triticain-α the negatively charged amino acid residues, glutamate 62 and aspartate 160, which can interact with the substrate. Sequence alignment of the protease domains of Triticain-α and other papain-like proteases detected variability of these residues.



The Michaelis constants presented here describe the kinetics of substrate/enzyme binding. As we can see, an increasing of pH leads to an intensification in the specificity of Triticain-α and its structural homolog Cathepsin L with respect to the peptide fluorogenic substrates QLLR, QLLK, QLLQ, QLLD. Neither Triticain-α nor Cathepsin L could digest QLLD at neutral pH unlike the mutants E62N and E62K of Triticain-α. Simultaneously, E62K mutant binded QLLR, QLLK, and QLLQ somewhat worse than the original enzyme.







Two fluoromethyl ketone (FMK) inhibitors Ac-PLVE-FMK and Ac-VLPE-FMK were designed for cysteine cathepsins. They were shown to inhibit human renal cancer cell migration. Docking into Cathepsin L structure revealed that peptides mainly occupy the hydrophobic binding pockets due to the Val and Leu side chains. The presented data would allow researchers to design selective pH-dependent inhibitors for human cysteine cathepsins modifying P1 amino acid residue in FMK-inhibitors which proved to be effective.

Conclusion

We state that S1 binding pocket defines specific pH-dependent recognition of substrates by PLCPs, ensuring multiple physiological functions of these proteases. Molecular docking in the crystal structure of Triticain-α revealed glutamate 62 and aspartate 160 in the S1 binding pocket, which can be responsible for such specificity through electrostatic interactions with a substrate. The obtained data might serve as a key for designing novel specific inhibitors for PLCPs.

Acknowledgements
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