IECBM 2022

MDPI

Abstract

pH-Dependent Specificity of Papain-Like Cysteine Proteases is Determined by S1 Binding Pocket †

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† Presented at the 2nd International Electronic Conference on Biomolecules: Biomacromolecules and the Modern World Challenges, 1–15 November 2022; Available online: https://iecbm2022.sciforum.net/.

Abstract: 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. This specificity is highly regulated by media conditions and the presence of accessory proteins. In this study, we examined structural aspects ensuring pH-dependent substrate specificity of PLCPs. Experiments employing fluorogenic peptide substrates demonstrated that plant PLCPs and human cathepsins possess a pH-dependent specificity for the residue in the P1 position. X-ray crystallographic studies and molecular simulations allowed overall structure determination of the enzymes to predict residues in the S1 binding pocket which can form electrostatic contacts with the substrates. Sequence analysis established variability of these residues among PLCPs. Based on the obtained data we designed a peptide inhibitor for human cathepsin L and described its inhibitory potential. As a conclusion, we state that the S1 binding pocket defines specific pH-dependent recognition of substrates by PLCPs, ensuring multiple physiological functions of these proteases. This work was supported by the Russian Science Foundation (grant No. 22-25-00648).

Keywords: papain-like cysteine proteases; cysteine cathepsin; enzymatic activity; substrate specificity; binding cleft

Citation: Petushkova, A.; Zalevsky, A.; Gorokhovets, N.; Makarov, V.; Savvateeva, L.; Serebryakova, M.; Golovin, A.; Zernii, E.; Zamyatnin, A. pH-Dependent Specificity of Papain-Like Cysteine Proteases is Determined by S1 Binding Pocket. *Biol. Life Sci. Forum* **2022**, *2*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s):

Published: date

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest: