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Abstract

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

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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 the 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 the 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 the 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 stated 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

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