Role for the S1 pocket in substrate specificity of papain-like cysteine proteases

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Papain-like cysteine proteases (PLCPs) are well-recognized as enzymes responsible for low-specificity digest of different proteins in lysosomes and other acidic cellular compartments. Growing evidence indicates that PLCPs have additional specific functions under ambient pH conditions characterized for the cytoplasm. In this study, we examined structural aspects ensuring pH-dependent substrate specificity of PLCPs. Mass-spectrometry analysis of the natural products of plant PLCPs-catalyzed digestion revealed that the enzymes cleave polypeptides with a hydrophobic residue in the P2 position. Meanwhile, experiments employing fluorogenic peptide substrates demonstrated that plant PLCPs and human cysteine cathepsins additionally 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 and 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 these data, we conclude that the S1 binding pocket may govern specific pH-dependent recognition of substrates by PLCPs, thereby ensuring multiple physiological functions of these enzymes. This work was supported by the Russian Science Foundation (grant No. 21-75-30020) and by the UK-Russia Young Medics Association.