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Investigation of the post-translational modifications effect on the activity of selected proteolytic enzymes

Post-translational modifications (PTMs) are responsible for covalent modifications of proteins. They occur after the translation process and significantly expand the pool of 20 natural amino acids. In this way, they influence the functions of proteins by changing their physical and chemical properties, thus increasing the natural diversity of the proteome. One of the common modifications is the attachment of a new functional group to the side chain of an amino acid residue, which may lead to a change in the chemical nature of the protein and constitute its regulatory mechanism protecting it against degradation by proteolytic enzymes. Research conducted in recent years has shown that e.g. phosphorylation of amino acid residues near the hydrolysis site (L-serine/L-threonine) can completely stop the hydrolysis of substrates by selected caspases.

The description of the research in this doctoral thesis can be divided into two parts. The first one concerned the design and synthesis of a chemical marker dedicated to cathepsin S, containing amino acid residues with post-translational modifications in its structure. For this purpose, substrate specificity profiles were determined at the P4-P2' position of human cathepsins B, L, V, S and K using the defined P1 library and the hybrid combinatorial substrate library (HyCoSuL). Both libraries contained a variety of unnatural amino acids, which allowed for the chemical space in the P4-P2' positions to be more extensively explored. The results obtained based on kinetic analyzes allowed the design of tetrapeptide fluorogenic substrates. Then the optimal substrate was converted into activity-based probe. For the synthesized chemical marker, the secondary inhibition constant (k_{obs}/I) was determined for all cathepsins. The obtained compound showed high selectivity towards cathepsin S.

The second part of the work focused on analyzing the impact of phosphorylation of serine and threonine residues in the P2-P2' positions on the activity of caspase-3, -7, -8 and -6. For this purpose, natural sequences and their analogues with phosphorylated residues from YAP1, VIME and PARP proteins were synthesized. Based on the kinetic analyses, it was shown that

that caspase-3 and caspase-7 have the ability to hydrolyze a sequence containing phosphorylated L-serine in the P1 position (ACC-βADEVpS↓GVK(Dnp)D), which is not observed in the case of the sequence with natural L-serine in P1 (ACC-βADEVpS↓GVK(Dnp)D). This result was also confirmed using liquid chromatography-mass spectrometry (LC-MS), which clearly confirmed the site of substrate hydrolysis. The work also shows that this hydrolysis does not occur in the case of a tetrapeptide substrate (Ac-DEVpS-ACC), which indicates the important role of sequence length and the appropriate adjustment of the peptide chain in the pockets of the discussed caspases.

Analyzing the substrate preferences of proteases using substrates containing amino acid residues with post-translational modifications may expand our knowledge of the biological functions of enzymes. The ability to hydrolyze such substrates may allow the determination of new functions of proteolytic enzymes. On the other hand, the observation of complete inhibition of hydrolysis may provide information about the regulatory mechanisms of given substrates in cells.