

Peptidyl-Resin Substrates as a Tool in the Analysis of Caspase Activity

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Rok wydania

2022

Czasopismo

Molecules

Numer woluminu

27

Strony

4107/1-4107/11

DOI

10.3390/molecules27134107

Kolekcja

Naukowa

Język

Angielski

Typ publikacji

Artykuł

Streszczenie

Caspases, proteolytic enzymes belonging to the group of cysteine proteases, play a crucial role in apoptosis. Understanding their activity and substrate specificity is extremely important.

Fluorescence-based approaches, including fluorogenic substrates, are generally used to confirm cleavage preferences. Here we present a new method of substrate specificity and activity analysis based on the application of fix-charge tagged peptides located on the resin. The proteolysis of peptide bond on the resin, occurring even with low efficiency, results in the formation of N-terminal fragments of model peptide containing ionization enhancers in the form of quaternary ammonium groups, allowing for ultrasensitive and reliable analysis by LC-MS/MS. The possibility of application of the proposed solution was tested through the analysis of substrate specificity and activity of caspase 3 or 7. The obtained results confirm the known substrate specificity of executioner caspases. Our solution also allowed us to observe that caspases can hydrolyze peptides shorter than those presented to date in the scientific literature.

Słowa kluczowe

caspases, substrate specificity, on-bead proteolysis, OBOC, fixed-charge tag, ESI-MS

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<http://dx.doi.org/10.3390/molecules27134107>

Strona internetowa wydawcy

<http://www.mdpi.com/journal/metals>