Application of *Safirinium N*-hydroxysuccinimide esters to derivatization of peptides for high-resolution mass spectrometry, tandem mass spectrometry, and fluorescent labeling of bacterial cells.

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Streszczenie

Mass spectrometry methods are commonly used in the identification of peptides and biomarkers. Due to a relatively low abundance of proteins in biological samples, there is a need for the development of novel derivatization methods that would improve MS detection limits. Hence, novel fluorescent N–hydroxysuccinimide esters of dihydro-[1,2,4]triazolo[4,3-a]pyridin-2-ium carboxylates (Safirinium P dyes) have been synthesized. The obtained compounds, which incorporate quaternary ammonium salt moieties, easily react with aliphatic amine groups of peptides, both in solution and on the solid support; thus, they can be applied for derivatization as ionization enhancers. Safirinium tagging experiments with ubiquitin hydrolysate revealed that the sequence coverage level was high (ca. 80%), and intensities of signals were enhanced up to 8-fold, which proves the applicability of the proposed tags in the bottom–up approach. The obtained results confirmed that the novel compounds enable the detection of trace amounts of peptides, and fixed positive charge within the tags results in high ionization efficiency. Moreover, Safirinium NHS esters have been utilized as imaging agents for fluorescent labeling and the microscopic visualization of living cells such as E. coli Top10 bacterial strain.

Słowa kluczowe

fluorescence microscopy, fluorescent probe, H/D exchange, live cell imaging, ionization tag, mass spectrometry, protein analysis, peptide labeling, tandem mass spectrometry