Direct detection of palmitoyl protein/peptide by mass spectrometry

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Abstract

Palmitoylation is a lipid modification of proteins with the covalent attachment of a palmitoyl group to a cysteine residue through a thioester linkage. Direction detection of palmitoylation is challenging because the thioester linkage is labile and palmitoyl group losses could occur during sample preparation if care is not taken. In addition, palmitoyl group dramatically increases the hydrophobicity of peptides, which makes them hard to elute using regular LC method for peptide. Here we present a sample preparation method and optimized LC-MS condition for direct identification of palmitoyl protein/peptide.

Three synthetic cysteine-containing peptides were palmitoylated via reaction with palmitoyl chloride in trifluoroacetic acid. The stability of the resulting palmitoyl peptides at different pH and temperatures with the presence of various reducing reagents was checked by MALDI-TOF mass. LC-MS experiments of palmitoyl peptides were performed on an LTQ-Orbitrap using in-house packed columns.

The palmitoyl peptides were incubated with different buffers (ammonium bicarbonate, Tris) at various concentrations and pH, with or without the presence of DTT or TCEP. The MALDI-TOF mass spectra showed no appreciable palmitoyl loss when palmitoyl peptides were incubated in 50mM Tris (pH 7.4) with TCEP (up to 10mM) at 37°C, whereas the presence of DTT accelerated depalmitoylation in all conditions.

LC-MS results showed that palmitoyl peptides were difficult to elute from C18 column commonly used for peptide separation, even with strong organic solvents such as isopropanol. Meanwhile, the palmitoyl peptides were retained on the C4 column through much weaker interaction, allowing elution and separation of palmitoyl peptides using mild solvents such as acetonitrile.

An optimized LC-MS condition and fragmentation behavior of palmitoyl peptides are currently underway and will also be presented.

This research is supported by NIH grants P41 RR010888, S10 RR015942, S10 RR025082, R01 GM078293, and contract N01 HV00239.