Electron capture dissociation mass spectrometric analysis of lysine-phosphorylated peptides.

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Streszczenie

Phosphorylation of proteins is an essential signalling mechanism in eukaryotic and prokaryotic cells. Although Nphosphorylation of basic amino acid is known for its importance in biological systems, it is still poorly explored in terms of products and mechanisms. In the present study, two MS fragmentation methods, ECD (electron-capture dissociation) and CID (collision-induced dissociation), were tested as tools for analysis of N-phosphorylation of three model peptides, RKRSRAE, RKRARKE and PLSRTLSVAAKK. The peptides were phosphorylated by reaction with monopotassium phosphoramidate. The results were confirmed by 1H NMR and 31P NMR studies. The ECD method was found useful for the localization of phosphorylation sites in unstable lysine-phosphorylated peptides. Its main advantage is a significant reduction of the neutral losses related to the phosphoramidate moiety. Moreover, the results indicate that the ECD–MS may be useful for analysis of regioselectivity of the N-phosphorylation reaction. Stabilities of the obtained lysine-phosphorylated peptides under various conditions were also tested.

Słowa kluczowe

electron capture dissociation, phosphorylation, phospholysine, post-translational modification