Identification Of Phosphorylated Peptides And Proteins From Mixtures Using Alternating Collision Induced Dissociation (CID) And Electron Transfer Dissociation (ETD) MS/MS

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Introduction

Post-translational modifications (PTM) of proteins, such as protein phosphorylation, play important roles in regulation of many cellular functions. The precise determination of the location of phosphorylation is crucial to the understanding of the regulation mechanisms. Mass spectrometry analysis combining database search has become the choice of many proteomics laboratories. However, these MS-based techniques are not without limitations and may produce false positive identifications due to insufficient amount of peptide sequence MS/MS information. Loss of phosphate group occurred preferentially in CID and could not provide information on the phosphorylation sites. McLafferty et. al., introduced an MS-based technique, electron capture dissociation (ECD) in 1968 which provided a way to obtain MS/MS spectra complimentary to CID data. Recently, a variation of the ECD technique has been developed and it has been shown to minimize false positives when using CID and database search for identification. The novel technique has been referred to as ETD which can provide similar results to the ECD process. An additional observation for the ETD technique is that the ion induced collision process is much gentler than the CID process and the phosphate remains attached to the amino acid during the collision process which allows the specific location of the phosphate to be observed.

Experimental

Materials

Angiotensin I and bovine serum albumin were purchased from Sigma (St. Louis, MI). The phosphopeptide TTHyGSLPQK was synthesized by SynPep (Dublin, CA). The phosphopeptide standard mixture was purchased from SynPep (Dublin, CA). Bovine serum albumin was digested with trypsin using a protocol based on 2:1 trifluoroacetic as the dehydrant. The digests were adjusted, dried and stored until use. HPLC grade solvents were obtained from D鸞ke & Jackson.

Results and Discussion

Chromatographic Analysis With ETD MS/MS

Figure 6. Base peak chromatogram of 10 fmol BSA tryptic digest analyzed by HPLC-Chip/MS with ETD MS/MS. The precursor preference is set to greater than doubly charged.

Figure 7. Spectrum Mill database search results of 10 fmol BSA tryptic digest analyzed using ETD. Seven peptides were identified, all of which were tryptophan.

Figure 8. ETD spectra of the phosphopeptides identified from the phosphopeptide standard mixture.

Conclusions

• ETD offers alternative tandem MS method and can be used in combination with CID
• ETD generates c and z ions which are complimentary to CID
• ETD allows localization of PTM
• ETD offers more complete sequence coverage for de novo sequencing