Identification of Unknown Modifications on Proteins During Database Searching

INTRODUCTION

Many software programs have been developed to identify proteins from mass spectrometric data. However, it is often difficult to identify a protein if it has been modified in some way, without prior knowledge of the possible modifications. The software programs Pro ID and Pro ICAT, as well as other programs that use the Interrogator database search algorithm, the Interrogator database search algorithm for identifying proteins from unidentified unidentified spectra, has the capability to search for a specific modification. At run time, Interrogator allows the user to input a 'zone modification' mass. Interrogator will consider any mass that results in the experimental mass differing from the expected mass of the peptide in the database. The Interrogator algorithm divides the reconstructed MS/MS data into sections or 'zones' and constructs hypotheses about which observed fragment ions should be ignored assuming that the observed precursor mass delta was localized to a particular zone. For each zone, a separate search is performed only the fragment masses that should be considered under the hypothesis. In the basic operation of Interrogator, the Interrogator search algorithm finds peptides within the subset that are likely matches and returns a specific modification mass, or the 'zone' or 'modification' to the database. This software is useful for identifying proteins from modified peptides.

RESULTS AND DISCUSSION

The Interrogator search algorithm works to find performing the Interrogator search database, Interrogator allows the user to search for a specific modification. At run time, Interrogator allows the user to input a 'zone modification' mass. Interrogator will consider any mass that results in the experimental mass differing from the expected mass of the peptide in the database. The Interrogator algorithm divides the reconstructed MS/MS data into sections or 'zones' and constructs hypotheses about which observed fragment ions should be ignored assuming that the observed precursor mass delta was localized to a particular zone. For each zone, a separate search is performed only the fragment masses that should be considered under the hypothesis. In the basic operation of Interrogator, the Interrogator search algorithm finds peptides within the subset that are likely matches and returns a specific modification mass, or the 'zone' or 'modification' to the database. This software is useful for identifying proteins from modified peptides.

MATERIALS AND METHODS

Samples: Protein tryptic digests were prepared from 2D-gels, MDLC, or using the ICAT technique (Applied Biosystems). MS/MS Spectra: • API QSTAR™: MALDI-TOF-TOF (Applied Biosystems) equipped with information dependent acquisition (IDA), nanoES (Protea) and capillary HPLC (Agilent or LC Packings). Databases: Data were analyzed using Pro ID or Pro ICAT software applications (Applied Biosystems) which automatically identify proteins from MS/MS spectra using the Interrogator database search algorithm (Pro ID software also quantitates ICAT reagent labeled peptides from the MS data). The NCBI non-redundant database or Swissprot database was searched.

TRADEMARKS/LICENSING

12. MPR: Interrogator and BioAnalysis are trademarks of Applied Biosystems and ICAT is a registered trademark of the Protein degradation, modified for the NEPHOS Group of Applied Biosystems. NCBI and SWISS-PROT are trademarks of NCBI. For further information, visit our website at:

REFERENCES