

AUTOMATED PSD ANALYSIS ON COMPLEX PEPTIDE MIXTURES

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Matrix assisted laser desorption ionisation mass spectrometry (MALDI MS) is an established method for protein identification based on the approach of peptide mass fingerprinting. At the simplest level, the products of proteolytic digestion of a target protein are analysed in linear time of flight (TOF) mode without prior purification or sample clean up. As only singly charged molecules are observed in MALDI MS, very complex mixtures can be analysed. The resulting mass signals are dependant on the sequence of the protein and therefore represent a unique pattern characteristic of the original target protein. The peptide masses can then be applied to any one of a number of Web-based search engines that use protein sequence databases in order to identify the protein¹.

With the increasing number of protein sequences that are being entered into the databases, as well as putative translations from DNA fragments, the ability for unambiguous identification has also decreased. Clarification can be obtained by including post source decay (PSD) fragment data, generated in reflectron TOF, from one or more parent ions detected in the peptide spectrum. These additional masses then allow unequivocal protein identification².

Generating PSD data, however, is generally viewed to be time- and sample-consuming, as fragment ions of different molecular weight need to be refocused to the detector to give a complete spectrum. A MALDI system, which employs both linear TOF and the unique curved field reflectron³ to generate parent ion spectra and seamless PSD spectra, respectively, is used to generate peptide and fragment data for database searching. Further, advances in ion gate technology (resolution ± 12.5 Da at 1000 Da) enabled selection of parent ions, from complex mixtures, so that the complete process of PSD analysis can be automated through software. Various stages of automation (automatic generation of parent ion spectra, automatic setting of high resolution ion gate to select parent for PSD, automatic generation of seamless PSD spectra with the curved field reflectron, automatic annotation of the PSD spectrum to enhance sequence interpretation) will be described and illustrated with examples of peptides generated from trypsin-digested proteins.

1. Cottrell, J. S.; Peptide Research **7**, 115 (1994)

2. Eng, J. K. *et al*; J. Am. Soc. Mass Spectrometry **5**, 976, (1994)

3. Cornish, T. J., Cotter, R. J.; Rapid Commun. Mass Spectrometry **8**, 781 (1994)