

AXIMA-QIT™

Characterization of Complex
Protein Gel Bands by Offline
LC-MALDI QIT TOF MS

- Combination of offline LC separation with MALDI QIT TOF MS
- Increased number of proteins identified from 1D gel band
- MS/MS data used for database searching for confident protein identification
- Immobilized sample permits increased time for acquisition and reduction in signal suppression

Characterization of Complex Protein Gel Bands by Offline LC-MALDI QIT TOF MS

Introduction

Traditionally, identification of complex protein mixtures has required a series of time-consuming protocols. Protein mixtures are separated using 1D or 2D gel electrophoresis, protein bands or spots excised and digested producing a peptide mass fingerprint (PMF) to identify the protein using MALDI mass spectrometry and database searching. Limitations can arise, including co-migration of more than one protein, suppression effects during ionization, or the PMF not correlating with any protein in the databases. In many cases, it is simply the complexity of the sample which may contain hundreds or thousands of tryptic peptides from a number of different proteins that proves to be the restrictive factor when attempting to assign putative protein identification.

It is clear that complicated samples require an added dimension of separation prior to MALDI analysis and the use of reverse phase liquid chromatography followed by deposition of the separated peptides directly onto a MALDI target has been recently reported. In this instance, the separated peptides are analyzed initially by MS techniques to provide a "candidate list" and subsequently by MS/MS which can provide sufficient information to generate useful sequence data and protein identification via database searching. Here, in an attempt to circumnavigate many of these issues we have analysed digested 1D gel samples using offline LC-MALDI QIT TOF MS.

Methods

Human proteins from BJAB (EBV-negative Burkitt's lymphoma) cell full lysate were grown in suspension in RPMI medium supplemented with 10% fetal calf serum, harvested and washed with PBS three times. The cell pellet was re-suspended in lysis buffer (1% CHAPS, 8.7% glycerol, 150 mM NaCl, 50mM Tris-HCl pH 7.4 and protease inhibitor cocktail). Following centrifugation, soluble proteins in the supernatant were recovered. The protein mixture was denatured (12.5 mM Tris-HCl pH 6.8, 4% glycerol, 0.4% SDS, 1% β-mercaptoethanol) and heated for 5 minutes at 95°C before loading on a 12% acrylamide 1-DE gel. After protein migration, the gel was stained with Coomassie brilliant blue G-250. From this gel, the 33-42 kDa region was excised and subjected to a tryptic digestion. Briefly, the gel slice was dried in neat acetonitrile before performing cysteine reduction and alkylation with iodoacetamide followed by trypsin digestion at 37°C overnight. After digestion, the supernatant was removed before a first extraction with 1% formic acid solution in water. This solution was pooled with the previous supernatant and a second extraction with 50% acetonitrile / 1% formic acid performed to increase peptide recovery.

Protein Identification - MS/MS Ions Mascot® Database Search
Phosphoglycerate kinase 1
Actin gamma
Aldolase A
SET protein (HLA-DR associated protein II)
Poly(rn) binding protein 1; heterogeneous nuclear ribonuclearprotein X
Laminin-binding protein
Translation elongation factor eEF-1 delta chain
Glyceraldehyde-3-phosphate dehydrogenase
HLA-A11K protein precursor
MHC class I histocompatibility antigen alpha chain
COP9 complex subunit 4
Heterogeneous nuclear riboprotein A2/B1
Cysteine and histidine-rich domain (CHORD)-containing
HRS
Aspartate aminotransferase 2 precursor, glutamic-oxaloacetic transaminase 2
Ribosomal protein P0, 60S acidic ribosomal protein P0
ProteinX0005
Thioredoxin-like 2
Heterogeneous nuclear ribonuclearprotein AB isoform b
Unknown gene product
Complement component 1, q subcomponent binding protein precursor
Phosphoserine aminotransferase, isoform 2
Isocitrate dehydrogenase 3 (NAD+) alpha precursor

Table 1

Figure 3. MS/MS of m/z 1446, identified as SET protein

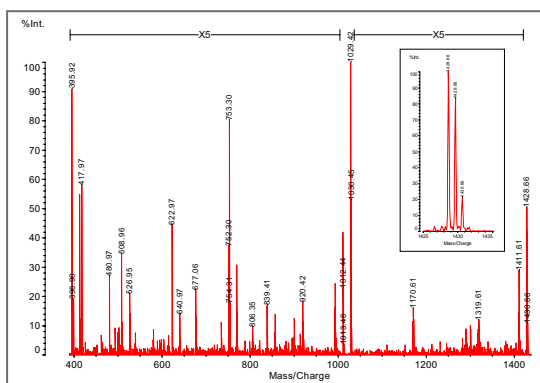


Figure 4. MS/MS of m/z 1848 with sequence tag annotated

