

AXIMA-QIT™

Investigating Rat Retinal Proteins
Using a MALDI TOF MS and
MALDI QIT TOF MS

- Investigation of the proteome of the rat retina using a curved field reflectron MALDI TOF MS and a novel MALDI QIT TOF MS
- CD based sample system used in conjunction with traditional MALDI sample preparation techniques
- Peptide mass fingerprinting and MS/MS experiments on a MALDI QIT TOF MS were used to identify proteins with high confidence

Investigating Rat Retinal Proteins Using a MALDI TOF MS and MALDI QIT TOF MS

Introduction

Several years ago an “ocular proteomics” project was initiated, the intention of which was to catalogue all of the proteins expressed in bovine retinas¹. Recently, a cataloguing effort on retinal proteins from mice and rats has been attempted. These rodents have been used extensively in laboratories as a model system and their genome projects are nearly completed, thus serving as excellent models for ocular proteomics studies.

Increasingly, large numbers of protein samples are being investigated and this large volume of samples in turn introduces a number of limiting steps in the work flow. One of these bottlenecks exists in the sample clean-up stage. It is commonplace to desalt proteolytic digest samples in order to increase signal-to-noise level and peptide sensitivity and reduce adduct formation. Pipette tips containing small beds of reverse phase packing materials may be used to perform this clean-up procedure acting as “mini-chromatography” columns onto which peptides are bound, washed and finally eluted. This can be time-consuming and labour intensive if performed manually.

A novel CD based system has recently been introduced that is capable of performing sample clean-up, concentration and elution onto an incorporated area specifically designed for MALDI acquisition. Advantages of this methodology include high reproducibility, increased sensitivity and reduced contamination. We have applied this new technology in combination with two MALDI based mass spectrometers for the identification of proteins from rat retinal extracts.

- The first series of analyses aimed to identify as many proteins as possible by peptide mass fingerprinting and database searching using both a curved field reflectron MALDI mass spectrometer and a MALDI QIT TOF MS.
- Additionally, identification of the protein samples was aided or confirmed by analysis on a novel MALDI-quadrupole ion trap - time of flight system providing important MS/MS information used to identify proteins by database searching fragmentation data.

Methods

Protein samples extracted from rat retina were processed by isoelectric focusing (IEF) gel and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) as previously described². The protein spots then underwent in-gel digestion with trypsin. The resultant digests were divided into two aliquots, dried down and stored at -20°C.

One set of samples was then reconstituted in 5 µl of 0.1 % TFA and processed on the GyroLab Workstation (Gyros AB, Uppsala, Sweden), resulting in a CD containing desalted tryptic digests deposited with alpha-cyanohydroxycinnamic acid matrix (1 mg/ml in 0.1% TFA/50% acetonitrile with 0.1 % octyl glucoside and 1 mg/ml fucose). The CD was introduced into the mass spectrometers using a specially designed MALDI target adaptor (Figure 1).

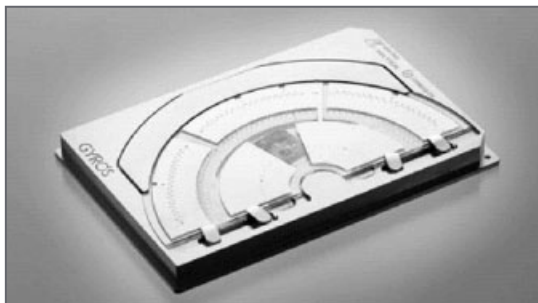


Figure 1. Gyros CD loaded with tryptic digests in MALDI target adaptor

The second set of samples was reconstituted in 5 µl 0.1% TFA, desalted and a 0.5 µl aliquot of each sample spotted directly onto a 384 well stainless steel MALDI target with an equal volume of dihydroxybenzoic acid matrix (12.5 mg/ml in 0.1% TFA/50% acetonitrile).

To generate peptide mass fingerprints, an AXIMA CFR™*plus* MALDI TOF mass spectrometer incorporating a curved field reflectron was used in positive ion reflectron mode. In addition, a novel hybrid mass spectrometer incorporating a MALDI ionization source, a quadrupole ion trap and a reflectron time-of-flight analyser³, the AXIMA QIT™ MALDI TOF was used to generate peptide mass fingerprints and MS/MS data for protein identity confirmation. Both instruments can accommodate the same target format and therefore samples could be analyzed on one system and subsequently on the alternative system without the need to re-prepare samples.

For all database searches, Mascot® (Matrix Science) was used on an internal server.

Results

Initially, samples were analyzed by MALDI mass spectrometry to provide tentative identification by peptide mass fingerprinting (PMF). The majority of samples were successfully identified using this simple method. An example of a typical PMF is shown in Figure 2. However, in some cases, unequivocal protein identification was not possible - this was due to a number of possible factors including few peptides in the sample or low protein concentration. In order to confirm identification, the samples were subjected to MS/MS analysis on an AXIMA QIT™ followed by database searching in an attempt to identify the protein.

The results from these experiments are summarized in Table 1 (see below).

A small minority of samples could not be identified by peptide mass fingerprinting alone, for example, samples 6, 10, 22 and 29. As described earlier, MS/MS was acquired on representative peptides from each sample. The MALDI QIT TOF system has the capability to select MALDI generated ions with high specificity and produce fragmentation spectra that may be database searched. The MALDI ion source, quadrupole ion trap and the time-of-flight analyzer are decoupled affording high resolution spectra independent of mode of operation, for example in both MS and MSⁿ modes. An example of the superior resolution obtained in both MS and MS/MS modes is shown below in Figure 3. MS/MS spectra for samples 6, 10 and 29 are shown in Figures 4-6.

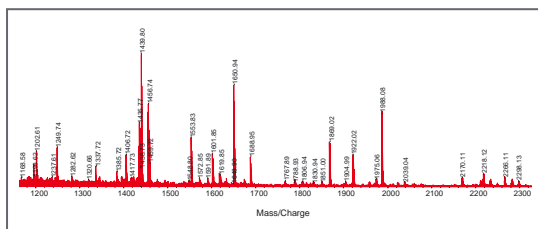


Figure 2. Typical peptide mass fingerprint from AXIMA QIT™ - sample 7

Spot Number	Candidate	MWt (Da)	Identified by PMF	Identified by MS/MS
1	Mitochondrial aconitase	85475	✓	✓
2	Transferrin	75859	-	✓
3	Albumin	68719	✓	✓
4	Transketolase	67644	✓	✓
5	Pyruvate kinase, M2 isozyme	57781	✓	✓
6	ATP synthase (H ⁺ transporting)		-	✓
7	ATP synthase β chain	51203	✓	✓
8	γ enolase	47141	✓	✓
9	Creatine kinase, B chain	42713	✓	✓
10	Enolase 1, α	47116	-	✓
11	α 1 actin precursor	42051	✓	✓
12	Glutamine synthetase	42268	✓	✓
13	Phosphoglycerate kinase 1	44555	✓	✓
14	Glutamate oxaloacetate transaminase 2, mitochondrial	47315	✓	✓
15	Guanine nucleotide-binding protein	37393	✓	✓
16	Glyceraldehyde-3-phosphate dehydrogenase	35828	✓	✓
17	Glyceraldehyde-3-phosphate dehydrogenase	35828	✓	✓
18	Malate dehydrogenase, mitochondrial	35656	✓	✓
19	Malate dehydrogenase-like enzyme	36483	✓	✓
20	Lactate dehydrogenase A	36451	✓	✓
21	Voltage dependent anion channel 1	30756	✓	✓
22	Phosphoglycerate mutase type B subunit	28846	-	✓
23	Triosephosphate isomerase 1	26921	✓	✓
24	Triosephosphate isomerase 1	26921	✓	✓
25	Fertility protein SP22	19974	✓	✓
26	Subunit d of mitochondrial H-ATP synthase	18782	✓	✓
27	Phosphatidylethanolamine binding protein	20802	✓	✓
28	Myosin, light polypeptide2, alkali	18970	✓	✓
29	Synuclein, beta	14495	-	✓
30	Low intensity peptide signals - no ID	-	-	-

Table 1

