Tandem Mass Spectrometry Analysis of Ex Vivo Amyloid Fibril and Tissue Samples

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OVERVIEW

Purpose
Various techniques were utilized to process and characterize the amyloid fibril as well as tissue samples. Utilizing the LTQ-Orbitrap for high performance online LC/MS^2, fast protein identification and post translational modifications can be achieved with minimum sample preparation steps.

Results:
Light chain sequence coverage was obtained with relative ease as well as many proteins be identified. LTQ-Orbitrap provided a powerful approach to characterize amyloid samples.

INTRODUCTION

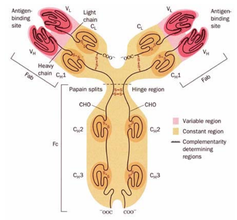
Amyloid fibrils are composed of abnormally folded proteins. Previously we have utilized multiple analytical methods to successfully characterize immunoglobulin light chain (Ig LC) proteins from the urine and blood) [1,2], whereas the highly insoluble fibrillar deposits in the tissues are more refractory to structural analysis) [3]. The studies reported here are focused on *ex vivo* amyloid fibrils and tissue samples from a patient with AL amyloidosis studied by various Mass Spectrometry techniques, Atomic Force Microscopy (AFM), and 1D and 2D gel electrophoresis. The LC-MS/MS mass spectrometer system enabled us to acquire more complete information for protein identification and determination of post-translational modifications, thus extending the results obtained during preliminary analyses that utilized MALDI-TOF MS and MALDI-Q-o-TOF MS.

METHODS

Using the water extraction method reported by Skinner *et al.*, amyloid fibrils were extracted from the organs of a patient who had succumbed to AL amyloidosis (AP 98-83) at the Boston University Medical Center. Tissue samples were directly obtained from the heart of the patient and were first homogenized, then either washed with PBS buffer before processed with PCT or directly processed with PCT without washing. The extracted protein samples were fast trypsin digested using the Pressure Biosystems PCT system. The fibril sample from the same patient was also processed as a control.

MS analyses of the digests were first carried out using a Reflex IV reflectron TOF MS (Bruker). Then they were analyzed by LC/MS^n using reversed phase chromatography on an Acuity nanoLC (Waters) interfaced to the LTQ-Orbitrap MS (Thermo-Fisher). The spectra were searched with an in-house Mascot with commercial and customized databases. The Mascot search results were imported into the Scaffold 2 Proteome Software bundled with X! Tandem for further analyisis.

Fig 1. Immunoglobulin Structure



IgG Light Chain ~ 214 AA

1 -108 Variable region
109 -214 Constant region

RESULTS

Figure 2. 2D gel- electrophoresis separation of the deposited proteins from AL fibrils in the patient's heart. There are clearly several bands in the gel which are spaced in a manner that is similar to the serial spots found in 2D-SDS-PAGE gels used for analysis of adipose fat tissue biopsies reported previously (Lavatelli *et al.*,[4] Figure 2)

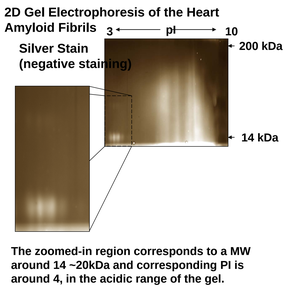


Fig 3. MALDI MS and AFM of Light Chain fibrils from different organs

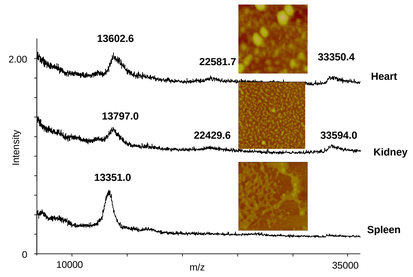


Fig 4. Protein concentration in samples extracted from homogenized Heart Tissue by processing with PCT.

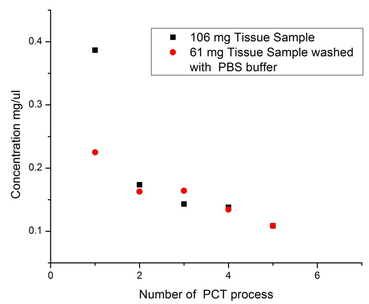


Fig 5. LC-MS/MS spectra of the $[M + 2H]^{2+}$ ions observed in the LC/MS analysis of trypsin-digested AL fibril samples from a patient's heart. A: $[M + 2H]^{2+}$ *m/z* 940.94, assigned to residues 1–18. B: $[M + 2H]^{2+}$ *m/z* 883.42, assigned to residues 2–18. Oxidation of Met4 is observed in both spectra shown here.

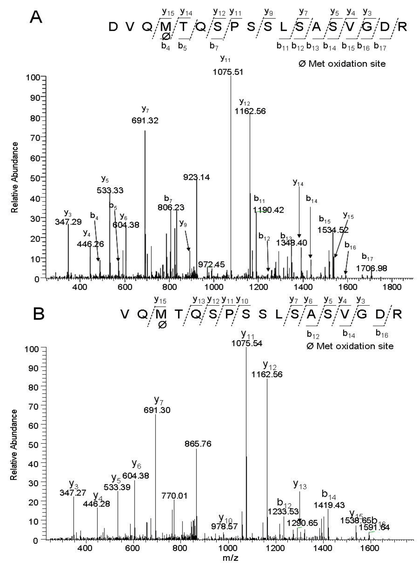


Figure 6. Error tolerant MASCOT search was performed on the cDNA-derived protein sequence in order to assign the LTQ-Orbitrap data. Combined peptide sequences assigned from tryptic digests of all samples are underlined in the theoretical AA sequence of the LC sequence predicted on the basis of the LC cDNA from the AL patient. The Met4 oxidation site and eleven truncation sites are marked.

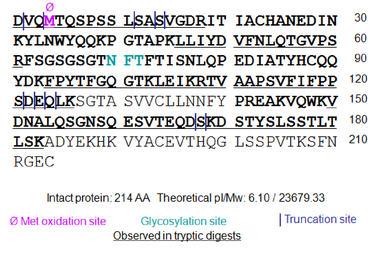


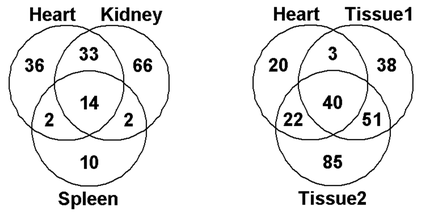
Table1. Sequence coverage of all the samples. Tissue 1 was homogenized heart sample without PBS buffer washing and Tissue 2 was with PBS buffer washing.

	Heart	Kidney	Spleen	Tissue1	Tissue2
Sequence coverage	31%	16%	22%	43%	30%

Table 2. Filtered list of the proteins identified from samples. Most redundant identifications have been removed. The identified IgG chain sequences are consistent with the cDNA-derived protein sequence.

Identified Proteins	Heart	Kidney	Spleen	Tissue1	Tissue2
Keratin, type II cytoskeletal 1	100%	100%	100%	100%	100%
Keratin, type I cytoskeletal 10	100%	99%	100%	100%	100%
Hemoglobin subunit beta	100%	100%	100%	100%	100%
Actin, alpha skeletal muscle	100%	100%	51%	100%	100%
Myosin-7	100%	0	0	100%	100%
ATP synthase subunit beta, mitochondrial	100%	100%	100%	100%	100%
ATP synthase subunit alpha, mitochondrial	100%	100%	100%	100%	100%
Hemoglobin subunit alpha	100%	100%	77%	100%	100%
Desmin	100%	0	0	100%	100%
Ig kappa chain V-I region Mev	74%	89%	0	92%	85%
Tropomyosin alpha-1 chain	100%	86%	0	100%	100%
Heat shock protein beta-1	100%	89%	77%	100%	100%
Fatty acid-binding protein, heart	0	0	0	100%	100%
Alpha-actinin-2	100%	0	0	100%	100%
Myosin light chain 3	100%	0	0	100%	100%
Serum albumin	98%	100%	0	100%	100%
Titin	100%	0	0	100%	100%
Myosin-binding protein C, cardiac-type	100%	0	0	100%	100%
Desmoplakin	0	0	0	100%	100%
Peroxiredoxin-1	56%	88%	0	100%	100%
Glyceraldehyde-3-phosphate dehydrogenase	100%	100%	100%	100%	100%
Collagen alpha-3(VI) chain	100%	100%	0	100%	100%
Histone H4	76%	100%	77%	100%	100%
Myoglobin	74%	0	0	100%	100%
Filamin-C	100%	0	0	75%	100%
Alpha-crystallin B chain	100%	86%	0	100%	100%
Hemoglobin subunit delta	0	0	0	100%	100%
Heat shock protein	100%	0	0	84%	100%
Cytochrome c oxidase subunit 2	100%	89%	0	79%	100%
Tubulin beta chain	100%	100%	0	71%	100%
Myosin regulatory light chain 2	100%	0	0	0	100%
Aspartate aminotransferase, cytoplasmic	100%	100%	0	100%	100%

Figure 7. The protein Venn Diagram of the search results



DISCUSSION

The mass spectra obtained for the fibril proteins and their tryptic peptides were similar to the spectra generated previously in our laboratory from the purified urinary LC from this patient, but the signal intensities were much lower. MS/MS spectra of the dominant peak in the fibrils confirmed that the major component was IgG LC that corresponds to the IgG light chain gene sequence deduced from gene analysis of the clonal plasma cells in the bone marrow of this patient.

The LC-MS^2 data generated from extractions of homogenized tissue without further treatment achieved the highest sequence coverage and provided more identified truncation sites than did analysis of purified LC and tissue washed with PBS buffer. More proteins were identified in the PBS-washed tissue sample than others. This is an interesting result that, with the high sensitivity of the LTQ-Orbitrap instrument, minimum sample preparation proved to be effective and, in fact, achieved better results.

The Mascot data base search revealed that the LC proteins had multiple PTMs, including various truncation sites, and other proteins co-precipitated with the AL LC. Some common proteins were found in all samples, *e.g.*, hemoglobin (which probably indicates some residual blood contamination), keratin and myosin. Some of these were previously identified in our adipose tissue biopsy data [4], *e.g.* Vimentin, Glycerol-3-P dehydrogenase. Other proteins known to be associated with amyloid deposits were found, *e.g.*, serum amyloid P-component (found in both heart and spleen samples but not in the kidney sample). ATP synthase subunit alpha was found in all three samples. The proteins found in all three AL samples included ATP synthase, heat shock proteins, histones, creatine kinase *etc*. The common factors may be critical in the formation of LC amyloid fibrils, whereas those which are found in only one or two AL samples may reflect differences in the physiological environments in different organs. Combining all these results, we have obtained a broad structural dataset which provides information on LC fibril formation, composition, truncation, and other PTMs.

CONCLUSIONS

(1) The combined PCT extraction, digestion and LTQ-Orbitrap MS-based analysis platform offers a convenient way to identify tissue proteins, as well as the IgG light chain sequence.

(2) Compared with the fibril results, the less-processed tissue data generated more sequence coverage, as well as allowing the determination of many truncation sites different from those found in fibril data.

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