iTRAQ Quantitation in an Ion Trap Mass Spectrometer for Synaptic Proteomics and O-GlcNAc Site Mapping in Human Alzheimer's Disease

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Overview

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Results
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   a. Optimum collision energy – 36%
   b. Optimum data acquisition method – Profile
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2. Quantitation of Proteins in Human AD synaptosomes
   a. Identified 187 proteins
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3. Identification of O-GlcNAc modification sites
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   b. Application of novel ETD fragmentation for O-GlcNAc site mapping (novel O-GlcNAc site mapped on Synapsin 1A)

Introduction

While linear ion trap mass spec is a sensitive tool for the identification of proteins, small molecules, and post-translational modifications, a limitation has been its use for quantitation with iTRAQ differential isotopic labeling is only now beginning to become feasible (Griffin, T.J., et al. 2007. J. Prot. Res. 6(11):4200). iTRAQ depends on analysis of low molecular weight reporter ions not usually observed in standard ion trap MS/MS. A novel form of fragmentation called Pulsed-Q dissociation (PQD) has been introduced to allow for detection of low mass iTRAQ reporter ions.

Human Alzheimer's disease (AD) is a neurological disorder accounting for roughly 60-80% of dementia cases and is characterized by the gradual cessation of memory formation and storage. The disease is characterized by extracellular amyloid β (Aβ) plaques and neurofibrilary tangles of the protein Tau, leading to synaptic deficits and neurodegeneration. Thus, synaptic proteomic changes in AD are of interest.

The post-translational modification O-linked β-N-acetylglucosamine (O-GlcNAc) has aslo been implicated in AD pathology. O-GlcNAc is a cytosolic carbohydrate modification that in many ways acts like phosphorylation in protein regulation. O-GlcNAc is enriched at neuronal synapses and is decreased in AD brains. In some cases O-GlcNAc can compete with phosphorylation. Reduced O-GlcNAc modification of Tau is implicated in Tau hyperphosphorylation leading to neurofibrillary tangles. Thus, identification of novel synaptic O-GlcNAc sites may be relevant to understanding AD synaptic deficits.

Here we describe iTRAQ optimization in an LTQ linear ion trap using PQD fragmentation and apply this technique to quantitating synaptic protein expression changes in human cortex synaptosome preparations from aged non-demented vs. AD patients. Several protein expression changes observed are consistent with defective synaptic signaling/neurodegeneration and protein folding. Additionally, we mapped several O-GlcNAc sites in vivo in the cortex tissue of human AD patients that predict regulation of proteins known to act critically in synaptic plasticity/memory formation.

Finally, an obstacle to O-GlcNAc site mapping has been the lability of the modification in standard CID. We demonstrate the utility of novel electron transfer dissociation (ETD) in identifying exact sites of O-GlcNAc modification, and use it to identify a novel O-GlcNAc site on the synaptic vesicle protein Synapsin IA.

Methods

A standard digest of casein protein was iTRAQ labeled in various ratios, and variables of ion trap LTQ PQD collision energies and profile vs. centroid data acquisition were tested. Human brain synaptosome preparations were digested with Trypsin and labeled with iTraq tags 114 (control) and 117 (AD). These peptides were fractionated by 2d SCX and RF LC-MS/MS coupled with LTQ liner ion trap mass spectrometry. Data was analyzed using iTracker software and mined differential protein expression changes in Human AD samples. Human cortex synaptosome tryptic digests were enriched for O-GlcNAc peptides by lectin WGA chromatogrphay and analyzed by LC-MS/MS using neutral loss of O-GlcNAc to trigger MS3. Purified Synapsin 1A was analyzed with electron-transfer dissociation (ETD) on a LTQ orbitrap.

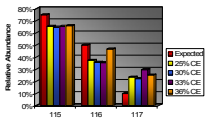


Chart 1. A collision energy of 36% is optimal for iTRAQ qunatitation of a standard casein digest labeled in a ratio of 10:7.5:5:1 114-117 iTRAQ tags.

Reference	Profile Data		Centroid Data	
	Average Ratio	Departure	Average Ratio	Departure
Casein alpha-S1	3.1297	0.6297	4.0882	1.5982
Casein alpha-S2	2.2711	-0.2289	2.9178	0.4178
Grand Total	2.8997	0.3997	3.7620	1.2820

Table 2. Data acquisition in profile mode provides more accurate iTRAQ quantitation that centroid mode.

Reference	0.20 Da Width	0.25 Da Width	0.30 Da Width	0.35 Da Width	0.40 Da Width
	Average of Ratio	Average of Ratio	Average of Ratio	Average of Ratio	Average of Ratio
Casein alpha-S1	2.3969	2.5846	2.6201	2.8427	2.9410
Casein alpha-S2	3.3498	2.8410	2.6862	2.5359	2.6554
Total	2.8734	2.7125	2.6531	2.6879	2.7982
Departure from expected	0.37	0.21	0.15	0.19	0.30

Table 3. iTraq labels 114:117 using a 2.5:1 ratio show 0.35 Da width of profile data is optimal for quantitation.

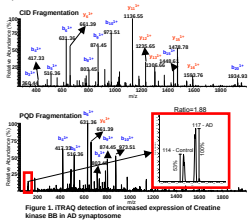


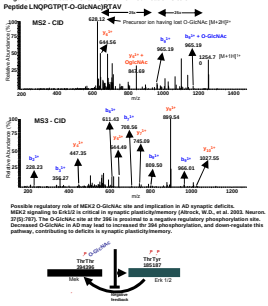
Figure 1. iTRAQ detection of increased expression of Creatine kinase BB in AD synaptosome

Results

Table 4. Proteins that were identified as differentially expressed in the cortex synaptosomes of human AD patients.

Up-regulated in AD	Accession Number	Average Ratio 117:114	Standard Deviation
EEA1, early endosome antigen 1, 162kD	NP_003557	6.4183	1.7638
SNAP25A, synaptosomal-associated protein 25	NP_003072	5.2406	1.4284
Ubiquitin carboxyl-terminal esterase L1	NP_004172	3.6410	0.5649
Aldehyde dehydrogenase 6A1 precursor	NP_005580	2.5858	1.0007
Glutaminase	NP_055720	2.7753	0.1269
N-Acetylglucosamine kinase	NP_060037	2.4640	0.1108
Guanine nucleotide binding protein alpha oB	AAM12609	2.4270	0.1476
cAMP-dependent PK cat. sub. β iso. 2	NP_002722	2.0702	0.4805
creatine kinase BB	NP_001814	1.8514	0.2061
Down-regulated in AD			
Heat shock 70kDa protein 2	NP_068814	0.4953	0.0198
P-type ATPase 11B	BAA76800	0.4868	0.1195
Phosphatidylinositol 3-kinase	BAB70696	0.4599	0.0208
Phosphoserine phosphatase	NP_004568	0.4507	0.2788
ATF/P1	AAH63855	0.4221	0.0372
Creatine kinase, mitochondrial 1B precursor	NP_066270	0.3994	0.1138
Heat Shock Protein 70kDa 12A	BAA24847	0.3231	0.0663
DnaJ (Hsp40) homolog, subfamily B, member 1	NP_006136	0.3067	0.0580

Figure 2. Identification of an O-GlcNAc modification site on MEK2
Peptide LNQPGTP(T-O-GlcNAc)RTAV



Possible regulatory role of MEK2 O-GlcNAc site and implication in AD synaptic deficits. MEK2 signaling to Erk1/2 is critical in synaptic plasticity/memory (Atkins, W.D., et al. 2003. Neuron. 37(6):767). The O-GlcNAc site at the 366 is proximal to a negative regulatory phosphorylation site. Decreased O-GlcNAc in AD may lead to increased thr 366 phosphorylation, and down-regulate this pathway, contributing to deficits in synaptic plasticity/memory.

Table 5. O-GlcNAc modified peptides identified in human AD samples.

Peptide	Reference
K.APII(S-O-GlcNAc)GVTK.A	Brain-specific protein p25 alpha (TPPP)
K.ITSNYEVIR.N	Piccolo
R.GLTQPTTVPA(T-O-GlcNAc)K.A	Bassoon protein
LNQPGTP(T-O-GlcNAc)RTAV	Mitogen-activated protein kinase kinase 2 (MEK2)
R.EEPLPT(T-O-GlcNAc)TPAAIK.E	Bassoon protein
R.APFQVTEDYTTK.G	Piccolo
SQSAAVTPSSTTSSTR	ADRM1

Bassoon and piccolo are essential for proper regulation of synaptic vesicle pools (Sun Y, et al. 2006. Mol Bio Cell. Epub May 25). ADRM1 is a novel proteasome subunit that binds the deubiquitinating enzyme UCH37 (Qui, XB., et al. 2006. EMBO. 25(24):5742). Indicating potential regulatory significance of O-GlcNAc on these proteins that when reduced in AD may contribute To synaptic deficits.

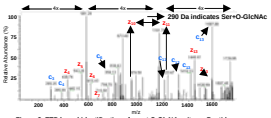


Figure 3. ETD based identification of exact O-GlcNAc site on Peptide GSHSQS(O-GlcNAc)SSPQALTLGR from Synapsin 1A on S436 residue.

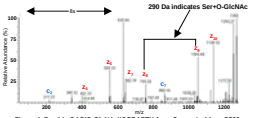


Figure 4. Peptide QAS(O-GlcNAc)SGPAPTK from Synapsin 1A on S568 residue from ETD analysis.

Conclusions

- Pulsed Q dissociation (PQD), when properly optimized, can be applied effectively in iTRAQ -based quantitation in ion trap mass spectrometry-based proteomics.
- Altered expression of synaptic proteins involved in synaptic vesicle release (e.g. SNAP25A), or in synaptic signaling linked to neuronal plasticity (e.g. cAMP PK) may contribute to synaptic deficits in AD. Altered expression in AD of HSP70, which has roles as an anti-aggregate molecular chaperone, may contribute to AD associated aggregation leading to build-up of senile plaques and tangles. Those protein expression changes need to be confirmed in additional comparisons of AD samples vs. control.
- Given that O-GlcNAc is reduced in AD, identification of O-GlcNAc modification sites on proteins that regulate signaling in neuronal plasticity (e.g. MEK2) and synaptic vesicle pools (e.g. Bassoon) may indicate possible pathological involvement in AD synaptic deficits.
- ETD is a valuable novel fragmentation strategy for exact site mapping of O-GlcNAc.