A Linear Ion Trap Method for Quantitative Proteomics: Analysis of Synaptic Membranes of Different Strains of Mice

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Overview

Purpose: To evaluate a novel ion activation technique, Pulsed-Q Dissociation (PQD), for its utility in quantitative proteomics.

Methods: Shotgun proteomics techniques were utilized using a Finnigan™ LTQ™ mass spectrometer enabled for PQD™ for quantitation of iTRAQ™-labeled synaptic membrane samples from 2 different strains of mice.

Results: High quality peptide, and hence protein, quantitation and identification was achieved.

Introduction

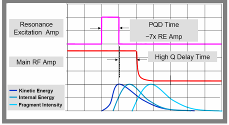
Traditionally ions are fragmented by resonance excitation CID in an ion trap such as the LTQ at a Q value of 0.25 or similar. This results in smaller m/z ions (fragment ions of less than 28% of the mass of the precursor) being unstable in the trap. Consequently, they are not detected. This makes the performance of proteomics experiments that rely on the detection of low mass ions difficult. Recently, Steve Carr's group has developed an MS3 [1] based approach. The focus of their research was to perform relative quantitation of proteomics samples using iTRAQ reagents and depended on Data-Dependent™ selection of predicted fragment ions generated by CID (such as the 291 m/z iTRAQ-labeled lysine y1 ion) for MS3 analysis. Another approach to extend the low mass range of linear ion traps for MS/MS has been developed and applied successfully to a complex proteomic sample here. This approach is referred to as Pulsed-Q Dissociation (PQD) and involves 3 discrete stages of operation of the linear ion trap: (1) activation of the precursor at high Q to allow precursors to gain kinetic energy rapidly, (2) a time delay at high Q to allow that kinetic energy to convert to internal energy through collisions and lastly (3) a pulse to low Q to trap all of the fragment ions generated after the collisions.

Methods

The PQD Process (Figure 1) :

1. Activation at High Q: PQD Time
2. Delay at High Q: HQD Time
3. Pulse to low Q and Trap fragments

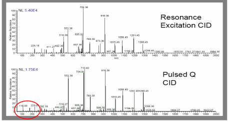
FIGURE 1. The PQD Process



The axes of Figure 1 represent time (x) in hundreds of microseconds and amplitude (y) in terms of volts. The PQD process involves three basic steps. First, the precursor ion is placed at a very high Q value, by raising the RF amplitude. This causes the ion to oscillate at a very high frequency, which will allow for high kinetic energies. Next, a high amplitude, short duration resonance excitation pulse is applied. The duration is typically 100 microseconds, with an amplitude approximately 7 times greater than is used in the normal CID method. (Normal is ~2 V, PQD is ~14 V). This resonance excitation pulse causes the precursor ion's amplitude of motion to grow and therefore rapidly gain kinetic energy. The resonance excitation signal is then turned off, but the kinetically excited ions are left at the high Q value (the high Q delay time) typically for 100 microseconds. During this time, the ions undergo energetic collisions with helium, which converts the precursor ion's kinetic energy into internal energy. Then, before significant fragmentation occurs, the RF amplitude is rapidly reduced to an arbitrarily low value, typically to a value which will trap ions at 50 m/z and above. While at this low value, the internally excited precursor ions will fragment, allowing all fragment ions, even at low m/z, to be trapped and analyzed.

Example Data: CID and PQD

FIGURE 2. Comparison of CID and PQD Data



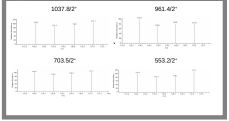
Typically, extra information is obtained in the low m/z region of the MS/MS spectrum.

Synaptic Membrane Preparations

The synaptic membrane fractions of two strains of mice (DBA2/J and C57/B6J) were purified, enzymatically digested and labeled with iTRAQ following the published protocol [2]. Duplicate, pooled samples (several individuals/label) were each labeled with iTRAQ reagents 114 and 115 (2 pooled DBA2/J mice samples) and 116 and 117 (2 pooled C57/B6J mice samples). Following the labeling, the 4 samples were further pooled and the peptides separated using strong cation exchange chromatography to produce 26 fractions which were then analysed by nano-LC-MS/MS on a Finnigan LTQ with PQD (Thermo Electron, San Jose). Fractions 9 to 11 were analyzed in triplicate. During data acquisition, each MS scan was followed by Data-Dependent acquisition of CID spectra of the 4 most intense precursors and by PQD of the same 4 precursors. PQD and CID data were merged and database searched using BioWorks (Thermo Electron, San Jose) against a mouse sequence database. Thus both PQD and CID contribute to the generation of protein identifications whereas it is PQD alone that contributes information (iTRAQ reporter ions) that allows the determination of relative protein quantitation.

Results

FIGURE 3. iTRAQ Labeling Reproducibility. Example Data for Synaptic Membrane Protein Calcium/Calmodulin-Dependent Protein Kinase Type Mouse



In this example it is clear that the ratio of the 2 strains of mouse is 1:1 (114 and 115 are DBA2/J and 116 and 117 are C57/B6J).

Five Example Proteins Displaying No Apparent Quantitative Difference between the Two Mouse Strains

A	B	D	E	F	G	I	J	K	L	N	O	P	Q
		Run 1				Run 2				Run 3			
Proteins	Peptide	114:114	115:114	116:114	117:114	114:114	115:114	116:114	117:114	114:114	115:114	116:114	117:114
ATPase, Na+K+ transporting, alpha 2 [Rattus norv		1.00	0.83	0.99	1.10	1.00	0.84	0.94	1.08	1.00	0.87	0.92	1.14
	R.L]NIPVSQVNPR.E	1.00	0.98	0.92	1.17	1.00	0.80	0.96	1.05	1.00	0.93	1.16	1.40
	K.N]MVPQQALVIR.E	1.00	0.69	1.03	1.11	1.00	0.89	0.70	1.03	1.00	0.91	0.66	1.08
	K.D]MTSEQLDEILR.D	1.00	0.70	0.98	1.11	1.00	0.73	0.87	1.01	1.00	0.81	0.81	1.08
	K.V]DNSSLTGESEPQTR.S	1.00	0.91	1.02	1.35	1.00	0.95	0.94	1.34	1.00	0.76	0.79	1.07
	K.G]VGIISEGNETVEDIAAR.L	1.00	0.88	1.01	0.77	1.00	0.82	1.22	0.99	1.00	0.93	1.18	1.13
A1A3_RAT Sodium/potassium-transporting ATPas		1.00	0.91	0.84	1.00	1.00	0.81	0.82	0.96	1.00	0.87	1.02	1.16
	R.D]VAGDASESALLK@.C	1.00	0.75	0.84	0.97	1.00	0.81	0.92	1.00	1.00	0.66	0.98	0.99
	K.E]AFQNAYLELGGLGER.V	1.00	1.22	1.33	1.36	1.00	0.88	0.78	0.87	1.00	0.76	0.91	1.12
	K.M]QVNAEEVVVGDLVEIK@.G	1.00	0.58	0.79	0.97	1.00	0.88	0.78	0.87	1.00	0.85	1.18	1.29
	R.T]VNDLEDSYGQQWTYEQR.K	1.00	1.07	0.40	0.82	1.00	0.68	0.88	1.09	1.00	1.22	1.02	1.31
malate dehydrogenase, mitochondrial [Mus musc		1.00	0.92	0.77	1.03	1.00	1.01	0.81	1.14	1.00	0.92	0.93	1.25
	K.I]FGVTTLDIVR.A	1.00	1.02	0.77	1.27	1.00	1.04	0.64	1.19	1.00	1.03	0.71	1.18
	K.G]C#DVVVIPAGVPR.K	1.00	0.79	0.64	0.81	1.00	1.21	1.00	1.36	1.00	0.82	1.08	1.05
	K.G]YLGPEQLPDC#LK@.G	1.00	0.87	0.84	0.98	1.00	0.74	0.81	0.98	1.00	0.80	0.88	0.95
	K.E]TEC#TYFSTPLLLGK@.K	1.00	1.26	0.95	1.04	1.00	1.04	0.79	1.05	1.00	0.82	0.86	0.77
ATP synthase, H+ transporting, mitochondrial F1 c		1.00	1.00	0.90	1.20	1.00	1.03	0.95	1.16	1.00	0.94	0.94	1.17
	R.V]LSIGDGIAR.V	1.00	1.02	0.92	1.19	1.00	0.92	0.75	1.08	1.00	0.84	0.85	1.14
	K.A]VDSLVPIGR.G	1.00	1.20	1.22	1.19	1.00	1.07	0.87	1.06	1.00	1.03	1.15	1.32
	K.T]GTAEMSSILEER.I	1.00	0.91	0.63	1.30	1.00	1.05	1.22	1.37	1.00	0.74	0.77	0.96
	R.I]LGADTSVDLEETGR.V	1.00	0.85	0.82	1.12	1.00	1.07	0.95	1.12	1.00	1.14	0.97	1.26
ATP synthase beta-subunit [Mus musculus]		1.00	1.08	0.97	1.11	1.00	1.14	0.92	1.09	1.00	1.05	1.01	1.27
	K.I]PVGPETLGR.I	1.00	0.88	0.80	0.90	1.00	1.28	1.10	1.33	1.00	1.13	1.04	1.32
	R.T]IAMDGTEGLVR.G	1.00	1.22	1.06	1.28	1.00	1.17	1.01	1.34	1.00	1.08	0.97	1.34
	R.I]MNVIGEPIDER.G	1.00	1.12	1.16	1.19	1.00	0.90	0.99	0.97	1.00	1.01	0.87	1.09
	R.F]TQAGSEVSALLGR.I	1.00	1.36	0.93	1.07	1.00	0.95	0.75	1.08	1.00	1.19	0.88	1.15
	R.V]ALTGLTVAEYFR.D	1.00	1.30	1.10	1.24	1.00	1.20	1.04	1.18	1.00	0.92	1.04	1.11
	K.T]VLIMELINNVAK@.A	1.00	0.78	0.75	0.94	1.00	1.36	0.92	0.94	1.00	1.11	1.05	1.41
	R.D]QEGQDVLLFIDNIFR.F	1.00	0.91	0.99	1.18	1.00	1.10	0.62	0.79	1.00	0.93	1.21	1.46

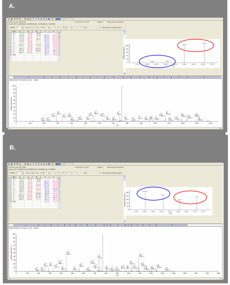
iTRAQ reporter ion ratios were:

1st protein:	**1 : 0.85 (0.02) : 0.95 (0.04) : 1.11 (0.03)**	**Av 0.93 : 1.03**
2nd protein:	**1 : 0.86 (0.05) : 0.89 (0.11) : 1.04 (0.11)**	**Av 0.93 to 0.96**
3rd protein:	**1 : 0.95 (0.05) : 0.84 (0.08) : 1.14 (0.11)**	**Av 0.98 to 0.99**
4th protein:	**1 : 0.99 (0.05) : 0.93 (0.03) : 1.18 (0.02)**	**Av 1.00 to 1.05**
5th protein:	**1 : 1.09 (0.05) : 0.97 (0.05) : 1.16 (0.10)**	**Av 1.05 to 1.06**

Since iTRAQ reporter ions 114 and 115 are duplicates for DBA2/J mice and 116 and 117 are duplicates for C57/B6J mice the ratios are in effect 1:1 for all 5 proteins within a limit of approximately 10%.

Acknowledgements

FIGURE 4. Example of Synaptic Membrane Quantitation Difference



In the PQD MS/MS spectrum (Fig. 4A) we see a clear example of a difference in peptide level between the 2 strains of mouse measured in a biological duplicate sample. In the bottom PQD MS/MS spectrum (Fig. 4B) we see an example of similar levels of a different peptide (but same protein) detected for the 2 mouse strains. These quantitative differences have been measured reproducibly in 3 separate LC-MS experiments. What is of particular interest here is that the peptides seemingly derive from the same protein. How can this be so since obviously they demonstrate different patterns of protein expression and therefore are likely performing different functions in the synapse? The PQD/CID MS/MS sequence evidence implies strongly that the 2 peptides derive from different splice variants of the same gene.

Conclusions

PQD is a promising new ion activation technique allowing the mass range of the linear ion trap for MS/MS to be extended to low m/z values. This allows for reproducible quantitation using iTRAQ labeling strategies. Software to enable automated quantitation using iTRAQ labeled samples is in development. This dataset will be extensively studied and the results reported at a later date.

References

[1] Chang B et al. Implementation of multiplexed iTRAQ quantitation tags on ion trap instruments. Proceedings of the 53rd ASMS Conference on Mass Spectrometry and Allied Topics; 2005 June5-9, San Antonio, Texas
[2] Ross PL, et al., Multiplexed protein quantitation in saccharomyces cerevisiae using amine-reactive isobaric tagging reagents Mol Cell Proteomics, 2004, 1154-69

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