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Correlation between y-Type Ions Observed in Ion Trap and Triple Quadrupole Mass Spectrometers

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Abstract

Multiple reaction monitoring mass spectrometry (MRM-MS) is a technique for high-sensitivity targeted analysis. In proteomics, MRM-MS can be used to monitor and quantify a peptide based on the production of expected fragment peaks from the selected peptide precursor ion. The choice of which fragment ions to monitor in order to achieve maximum sensitivity in MRM-MS can potentially be guided by existing MS/MS spectra. However, because the majority of discovery experiments are performed on ion trap platforms, there is concern in the field regarding the generalizability of these spectra to MRM-MS on a triple quadrupole instrument. In light of this concern, many operators perform an optimization step to determine the most intense fragments for a target peptide on a triple quadrupole mass spectrometer. We have addressed this issue by targeting, on a triple quadrupole, the top six y-ion peaks from ion trap-derived consensus library spectra for 258 doubly charged peptides from three different sample sets and quantifying the observed elution curves. This analysis revealed a strong correlation between the y-ion peak rank order and relative intensity across platforms. This suggests that y-type ions obtained from ion trap-based library spectra are well-suited for generating MRM-MS assays for triple quadrupoles and that optimization is not required for each target peptide.

Keywords

multiple reaction monitoring (MRM); selective reaction monitoring (SRM); triple quadrupole; ion trap; mass spectrometer; y-ions; spectral library; spectral correlation

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Supporting Information Available: Supplemental Table 1, a description of the contents of the ISB standard 18 protein mix; Supplemental Table 2, the tabulated AUC and peak intensity data for the full combined data set; Supplemental Figure 1, the results for the rank order permutation test for the three individual data sets; Supplemental Figure 2, the results from the relative intensity randomization test for the three individual data sets; Supplemental Figure 3, the results from an alternative relative intensity randomization test in which the triple quadrupole spectra were randomized by permuting the existing relative intensities for each spectrum; Supplemental Figure 4, the results from a second alternative relative intensity randomization test in which the triple quadrupole spectra were randomized by replacing each peak intensity with a random value from the data set; and Supplemental Analysis, the secondary rank order analysis performed on the 142 excluded peptides. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Introduction

Multiple reaction monitoring (MRM) is a targeted method of mass spectrometry (MS) allowing for the detection and quantification of specific molecules in a complex mixture. MRM-MS has been viewed as a viable alternative to affinity-based methods for biomarker verification because of its high sensitivity, dynamic range, and throughput.^{1,2} MRM-MS has been used for targeting small molecules in the pharmaceutical industry,^{3–5} drug metabolites in the clinical setting,^{6,7} and phosphopeptides.⁸ Recent reports include quantitative measurements in human serum with limits of detection reported in the nanogram per milliliter (ng/mL) to subnanogram per milliliter (subng/mL) range.^{9,10}

MRM-MS employs tandem quadrupoles to select for specified transitions from precursor to product ions. Specifically, a given precursor peptide ion is selected based on its mass-to-charge ratio (m/z) by the first mass filter, quadrupole (Q) 1, and fragmented by collision-induced dissociation (CID) in Q2. The resulting product ion fragments are then transferred through the second mass filter, Q3, which selectively detects fragment ions at specified mass-to-charge ratios. Because of the high selectivity of MRM-MS and the high duty cycle afforded by the ion beam nature of the method, it is capable of considerable sensitivity and multiplexing. Newly released instrument firmware incorporating scheduling algorithms are capable of performing experiments with in excess of 1000 transitions.^{10,11}

The selection of MRM transitions can potentially be guided by spectral information obtained during shotgun proteomic analyses. Large collections of shotgun data have been assembled into libraries for general use which could potentially provide a rich resource for designing MRM-MS experiments.^{12–14} In addition, a number of spectral library searching and building algorithms have been published that allow the development of consensus spectra from the many instances of MS/MS acquisition of a single peptide that exist in these libraries.^{15–18} One stumbling block to the transfer of spectral library-derived MRM transitions to actual MRM experiments, however, is the differences in instrumentation used for the two types of mass spectrometry. While nearly all discovery runs used to populate the spectral libraries are performed using ion trap platforms, due to their high sampling speeds and sensitivity, MRM experiments are performed using triple quadrupole instruments.

Low-energy peptide fragmentation by CID in triple quadrupole and ion trap mass spectrometers differ in mechanism, though both have been used extensively for proteomic applications. In CID performed by a triple quadrupole, energy is imparted to a beam of the selected ion which is subjected to collisional excitation during its passage through the second quadrupole, which is filled with a low-pressure gas. In this configuration, the fragment ions formed are frequently subjected to secondary fragmentation during their passage. It is well-known that b-ion signal from MS/MS spectra of tryptic peptides is typically weak when CID is performed in a quadrupole, while y-ion signal is comparatively robust.¹⁹ It has been postulated that this behavior results from facile secondary fragmentation of b-type ions that reduces the higher m/z peaks and increases the lower m/z peaks, while y-type ions are stabilized through proton sequestration by the carboxy terminal basic residue side chains.^{19,20} Fragmentation in an ion trap mass spectrometer takes place through on-resonance excitation, in which an isolated precursor ion is excited by applying a small alternating voltage across the end-caps that is m/z dependent such that product ions, once formed, are not subjected to ion acceleration.²¹ Hence, secondary fragmentation of b-type ions in an ion trap is reduced compared to that in a quadrupole.

Low-energy CID fragmentation of tryptic peptides is also governed by sequence-specific properties. In both instrument types, CID is typically dominated by cleavages along the amide backbone, resulting in the formation of a series of overlapping b- and y-type ions.^{19,22–24}

During CID, the cleavage of peptide bonds is typically governed by the mobility of a proton added during ionization, as explained by the mobile proton model.^{25,26} During collision-induced dissociation, the proton affinities of the peptide's side chains determine the most stable locations for the available protons^{27,28} and thus profoundly affect proton mobility and by inference the likelihood of fragmentation of a particular amide bond. Other effects have been described linking peptide sequence to dominant spectral ions: cleavages C-terminal to acidic residues dominate the spectra of peptides that have a localized proton, and cleavages N-terminal to proline dominate the spectra of those that have a mobile or partially mobile proton.^{29,30} Enhanced cleavages C-terminal to branched aliphatic residues (Ile, Val, Leu) are observed for y-ions from peptides that have both a mobile proton as well as a partially mobile proton.²⁹ The generation of neutral loss fragments is affected by proton mobility and amino terminal residues. Taken together, these data indicate that peptide sequence will profoundly affect y-type ion abundances independent of instrument type.

To determine the applicability of ion trap-derived consensus spectral libraries to the development of MRM assays for triple quadrupole-enabled experiments, we performed a correlation analysis of ion trap library spectra and their corresponding triple quadrupole-targeted MRM transitions. Given the known triple quadrupole bias toward y-ions discussed above, we restricted the analysis to this subset of fragments. Using two sets of peptides from a fractionated yeast extract, as well as one set from a digest of a standard protein mixture, we have found a very close correlation between the spectra generated on both instrument types, implying that spectral libraries will serve a valuable resource for targeted analysis by MRM.

Methods

The workflow employed for our analysis is summarized by Figure 1. The specific experimental details pertaining to the sample preparation, MRM list creation, mass spectrometry, data analysis, and statistical analysis are described below.

Sample Preparation. Yeast Extract Preparation

Yeast samples were prepared as described.^{32,33} Briefly, *Saccharomyces cerevisiae* strain (strain BY4741) was grown to an OD₆₀₀ of 1.0 in YPD. Cells were frozen in liquid nitrogen and disrupted with a Retsch PM100 mixer mill. Powder was suspended in 25 mM ammonium bicarbonate and cleared by centrifugation. Proteins were alkylated using iodoacetamide, digested overnight with trypsin (Promega, Madison, WI), and dried in a low pressure centrifuge. The sample was suspended in 1 mL of 0.1% formic acid in water. The sample was loaded to a C₁₈ cleanup column (Waters, Milford, MA), washed with the loading buffer, and eluted in 80% acetonitrile (ACN) and 0.1% formic acid. After drying, 500 µg of the sample was suspended in water and separated using the OFF-GEL Fractionator (Agilent, Santa Clara, CA) per the manufacturer's instructions, using a linear IPG strip with pH range 3–10. Fractions were then cleaned using C₁₈ Ultramicrospin columns (The Nest Group, Southborough, MA). Each fraction was analyzed on an LTQ (Thermo Scientific, Waltham, MA) for peptide content. Two fractions were chosen for analysis, one with pI range 4.37–4.87 (low pI fraction) and the other 8.16–9.13 (high pI fraction). The peptide pI values were calculated via the Trans-Proteomic Pipeline, which employs the ExPASy algorithm for determining pI based on amino acid sequence.^{34–36}

ISB Standard Protein Mixture Preparation

A mixture of 18 proteins ("18 mix", Supplemental Table 1) was prepared as described.³⁷ Briefly, 1 nmol of each protein was dissolved in 20 mM, pH 8.0, ammonium bicarbonate with 0.05% SDS, reduced with 2.5 mM TCEP at 50 °C for 30 min, and alkylated for 1 h with 10 mM iodoacetamide. The proteins were then digested by overnight incubation at 37 °C with

sequencing-grade trypsin (Promega) at a 1:40 (w/w) ratio. Samples were dried by centrifugal evaporation and cleaned up using a Waters Oasis MCX cartridge per the manufacturer's instructions. The final eluate was evaporated and resuspended in 1 mL of 0.1% formic acid and 1% ACN, in HPLC-grade water (VWR, West Chester, PA).

MRM Transition List Creation with MaRiMba

Using a software application called MaRiMba (manuscript under review), we generated an MRM transition list for each of the three samples of interest: yeast extract, low pI fraction; yeast extract, high pI fraction; and standard 18 protein mixture. For each of the yeast fractions, MaRiMba was instructed to build a list of MRM targets from a yeast spectral library provided by the National Institute of Standards and Technology (NIST) [NIST_ yeast_IT_v2.0_2008-07-11.splib from <http://www.peptideatlas.org/specplib/>] and to restrict each list to transitions corresponding only to peptides that had been observed previously in the LTQ shotgun analysis of the given fraction. For the 18 mix, a custom spectral library built from a publicly available and previously published ISB standard protein mixture data set³⁷ (http://regis-web.systemsbio.org/PublicDatasets/18_Mix/Mix_7/LTQ/) was used as the input to MaRiMba, with no further protein restriction (spectral library available upon request). Briefly, MaRiMba calls the program SpectraST^{16,18} to build a library of consensus spectra from the input spectral library and to generate a putative MRM list using a transition selection algorithm that chooses the highest quality transitions for each peptide spectrum. MaRiMba then filters the list according to user-specified criteria to generate a customized list of MRM transitions suitable for targeting. Here, all lists were filtered to include only singly charged y-ion fragments greater than 3 amino acids in length generated from doubly charged precursor peptides with m/z between 300 and 1600 and no methionine residues, no N-terminal glutamine residues, and no modified residues except for carbamidomethyl cysteine. Although triply charged peptides are also common products of electrospray ionization, the analysis was restricted to doubly charged peptides because our laboratory currently has developed well-optimized instrument parameters for only this class of peptides, which are necessary for achieving the maximal sensitivity needed to conduct an unbiased spectral correlation analysis. The two yeast extract lists also excluded peptides outside of the pI ranges of interest, 4.37–4.87 for the low pI fraction and 8.16–9.13 for the high pI fraction. Using the MRM lists generated by MaRiMba, we extracted the six most intense y-type ions from each peptide and used these as the targets for the MRM-MS analyses. In total, 400 peptides (2400 transitions) were targeted, including 225 peptides (1350 transition) from the low-pI yeast fraction, 134 peptides (804 transitions) from the high-pI yeast fraction, and 41 peptides (246 transitions) from the standard 18 protein mixture.

Mass Spectrometry

The two yeast MRM transition lists were each split into two MS runs to ensure high-quality, quantifiable elution curves would be acquired, while the 18 mix MRM transitions were targeted in a single run. Each of the five total runs was time-segmented by expected retention time to facilitate scheduling. For each run, a total of 32 overlapping time segments were used, with up to 32 transitions per time segment (i.e., the maximum degree of segmentation allowed by the instrument) and a retention time window of 1–3.5 min. The dwell time for each transition was constant throughout each run, but varied among runs from 5 to 15 ms. All runs employed a 5-ms interscan delay, a 5-ms interchannel delay, and a programmed scan width of 0 Da (corresponding to a realized scan width of 1 Da). For each MRM-MS run, 2.0 μ L of the given sample was loaded, corresponding to approximately 1 μ g of protein.

Data were acquired on a Waters Quattro Premier triple quadrupole coupled with a Waters nanoAcquity UltraPerformance LC (UPLC) pump fitted with a Waters Symmetry 5 μ m particle diameter C₁₈ 180 μ m $\times$ 20 mm trap column and a 1.7 μ m particle BEH130 C₁₈ 100 μ m $\times$ 100

mm analytical column. After loading and washing for 5 min with 0.1% formic acid in H₂O (buffer A), peptides were eluted using a linear gradient of 1–35% 0.1% formic acid in ACN (buffer B) over 30 min at a flow rate of 300 nL/min. For all runs, the MS instrument was operated in the positive mode. MS source conditions were evaluated for best response under positive mode nanoESI conditions by infusion of a standard solution via a syringe pump. MS source parameters were as follows: capillary voltage, 2.9 kV; cone voltage, 36 V; source temperature, 90 °C; and cone gas flow rate, 40 L/h at 4 psi. Nitrogen (99.998% purity, Airgas, Seattle, WA) and argon (99.999% purity, Airgas) were used as the cone and collision gases, respectively. Data acquisition was carried out by Masslynx V4.1 software.

Data Analysis

Data were converted to the mzXML format using Wolf-MRM (<http://tools.proteomecenter.org/software/wolf-mrm/wolf-mrm.zip>) and evaluated using the MRM analysis software MRMer³² (<http://proteomics.fhcrc.org/CPL/MRMer.html>). This tool was used to calculate and review the area under the curve (AUC) of each transition elution peak. Any peptide with transitions displaying poor coelution, low intensity, jagged peak shape, or otherwise flawed qualities that would impede reliable AUC quantification was eliminated from the analysis. For each accepted transition, the AUC value was taken as a measure of relative intensity and used for comparison to the corresponding peak intensity from the given ion trap consensus spectrum. In total, 258 peptides (1548 transitions) were included in the final analysis, which comprised 133 peptides (798 transition) from the low-pI yeast fraction, 96 peptides (576 transitions) from the high-pI yeast fraction, and 29 peptides (174 transitions) from the standard 18 protein mixture. Although a large portion of the originally targeted peptides were excluded from the analysis (142 of 400), this was done to ensure only accurate AUC values were used and thereby maintain the integrity of the analysis. A secondary analysis based on the information available for the excluded peptides (see Supporting Information) suggests that these peptides do follow similar trends to the included peptides, confirming that the analysis presented here is not biased because of the strict exclusion.

Statistical Analysis

To test the correlation between the MS/MS spectra derived from the ion trap-based spectral library and the transitions targeted on the triple quadrupole mass spectrometer, the AUC calculations for each MRM transition for a given peptide were compared to the corresponding ion trap library peak intensities. These comparisons were made in terms of two properties: (1) rank order, that is, the order in which a set of transitions rank when sorted by intensity, with no. 1 being the most intense; and (2) relative intensity, that is, the intensity of a given transition relative to that of the no. 1-ranking transition of the given peptide. All of the analyses described below were performed on each of the three data sets: low-pI yeast extract, high-pI yeast extract, and 18 mix.

Rank Order Analysis

To determine the rank order association between instruments, we considered the relationship between the ion trap (IT) rank number and the triple quadrupole (QQQ) rank number of each fragment peak. In particular, we charted the frequency with which the fragment peaks assumed each of the 36 possible rank order combinations, that is, IT rank 1 and QQQ rank 1, IT rank 1 and QQQ rank 2, IT rank 1 and QQQ rank 3, and so forth. In the resulting chart (see Figure 2), peaks that have the same rank for both instruments fall along the diagonal, while increasing disparity between ranks is reflected by increased distance from the diagonal. The rank order association was then assessed by the number of fragment peaks with the same rank for both instruments (“matching ranks”) and the number of fragment peaks with at most a one-rank difference between instruments (“within-one ranks”).

Rank Order Permutation Test

To test the statistical significance of the rank order association between instruments, we performed a permutation test to generate random data sets for comparison to the experimental data set, preserving the dependence structure of the ranks within each peptide. Specifically, we:

1. randomly permuted the rank order of the transitions from the triple quadrupole instrument for each peptide. The permutations were performed independently for each peptide.
2. calculated the number peaks with matching ranks in the corresponding (but nonpermuted) ion trap spectrum. This is equivalent to creating a rank order combination chart, as in Figure 2, and summing the counts along the main diagonal.
3. calculated the number of peaks with within-one ranks in the corresponding (but nonpermuted) ion trap spectrum. This is equivalent to summing the counts along the main diagonal $\pm$ one box in the rank order combination chart.
4. repeated (1)–(3) 10 000 times.
5. calculated a p -value for the correlation of the triple quadrupole and ion trap spectra in terms of matching ranks. This p -value was defined as the fraction of the 10 000 simulated data sets for which the number of peaks with matching ranks from the two instruments exceeded the number of peaks with matching ranks in the real data set.
6. calculated a p -value for the correlation of the triple quadrupole and ion trap spectra in terms of fragment peaks with similar ranks. This p -value was defined as the fraction of the 10 000 simulated data sets for which the number of within-one ranks exceeded this count in the real data set.

Relative Intensity Analysis

The correlation between the relative intensities of the fragment ions from the two instruments was assessed via dot product calculation. Specifically, each set of relative intensities for a given peptide from a given instrument was treated as a six-dimensional vector and normalized to the magnitude of the vector. For each peptide, the dot product was then calculated between the two normalized intensity vectors originating from the two different instruments, creating a distribution of dot product values for the data set. Since a dot product mathematically describes the similarity of two vectors, with a value of zero indicating complete orthogonality and a value of unity indicating identical vectors, these dot product calculations provided a quantitative measure of the similarity of the spectra from the two mass spectrometers.

Relative Intensity Randomization Test

To test whether the distribution of dot products indicated a significant correlation between the ion trap and triple quadrupole spectra, we performed a randomization test in which dot products were calculated, as described above, for a data set with randomized relative intensities and compared to the dot products for the observed spectra. Specifically, we:

1. replaced the triple quadrupole relative intensities for the fragment ions from a given peptide with the relative intensities for the fragments of a different, randomly selected peptide from the data set.
2. left the corresponding ion trap spectrum unchanged.
3. calculated the normalized dot product between the ion trap spectrum and the randomized triple quadrupole spectrum.

4. repeated (1)–(3) for each peptide.
5. plotted the distribution of normalized dot products for this random data set and compared this to the distribution for the experimentally observed data.
6. tested whether the experimental and random distributions were stochastically similar or distinct using the Wilcoxon rank-sum test. This test was used to (i) combine the dot products from the two distributions, (ii) calculate the order of each value, and (iii) define W as sum of these orders for all data points from the randomized sample. The p -value was determined by comparing W to the probability distribution of the Wilcoxon rank-sum statistic.

Results and Discussion

To address the concern of the generalizability of ion trap-derived consensus spectra to MRM experiments, we analyzed the correlation of the top six y -type ions of spectra obtained from a consensus spectral library built from ion trap data with the corresponding MRM transitions targeted on a triple quadrupole during this analysis. In particular, the intensity value for each of these fragment ions, as recorded in the consensus spectral library, was compared to the actual intensity of the corresponding transition measured on our triple quadrupole, as determined by AUC calculation of the transition elution peak. To avoid artifactual effects specific to particular peptide sequences, samples were chosen from low- and high- pI fractions of a tryptic digest of yeast separated by isoelectric focusing as well as a digest of a standard mixture of 18 proteins. Because the analyses yielded the same trends for all three data sets, they were collapsed into one combined data set, the results for which are presented here. Results from the individual data sets are provided in the Supporting Information, unless otherwise noted.

In this work, we followed the general workflow diagrammed in Figure 1. For each of the three samples included in the analysis, an MRM transition list was created from an appropriate ion trap-derived consensus spectral library using the software tool MaRiMba. For the two yeast fractions, the spectral library used was a comprehensive library for the yeast proteome created by NIST and provided for free download by Peptide-Atlas. To restrict our analysis to only the well-observed peptides from each yeast fraction, the MRM lists were limited to only peptides seen in our shotgun MS analyses of the given fractions. The 18 mix MRM list was generated from a custom-built spectral library of publicly available ion trap data³⁷ assembled using SpectraST. For each peptide in these data sets, information for the six most intense y -type ions was extracted including peak intensity, precursor peptide m/z , and product ion m/z . These m/z values were used to program MRM analyses for the three samples on a Waters Quattro Premier using transition scheduling, as described above. The raw data was analyzed using the MRM analysis tool MRMer³² to calculate AUC values for each triple quadrupole-targeted transition. The extracted AUC values were added to a database containing the corresponding spectral library-derived peak intensities. An excerpt of this tabulated data is given in Table 1, while the full data set, which includes information for a total of 258 peptides and 1548 transitions, is provided in Supplemental Table 2.

Rank Order of y -Type Ions Is Highly Persevered Across Ion Trap and Triple Quadrupole Instruments

To assess the qualitative similarity of the IT and QQQ spectra, the library peak intensities and transition AUC values were compared in terms of their rank orders in their respective peptide spectra. In particular, we charted the frequency with which the fragment peaks assumed each of the 36 possible rank order combinations, that is, IT rank 1 and QQQ rank 1, IT rank 1 and QQQ rank 2, IT rank 1 and QQQ rank 3, and so forth. This analysis revealed that the AUC values of the six targeted y -ion transitions for each peptide yielded the same or similar rank

orders as the intensity values of the corresponding fragment peaks in the ion trap-generated library spectra. This is illustrated in Figure 2, which depicts the rank order frequency chart described above. In this figure, charts are provided for the individual data sets, as well as the combined data set, to illustrate that the same trends are observed for all. It is clear from this data that the rank order of most spectra from the ion trap instrument agree well with those from the triple quadrupole, with 48% of all entries lying on the diagonal (matching perfectly) and 82% falling on the diagonal or in a neighboring box (matching perfectly or off by exactly one rank). These high percentages of matching and nearly matching ranks support the assertion that ion trap and triple quadrupole spectra are qualitatively very similar for y-type ions.

To test whether this observed rank order correlation was statistically significant, we generated 10 000 random data sets for comparison to the experimental data set using the rank order permutation test described above. Each randomized data set was created by randomly permuting the triple quadrupole ranks for each peptide spectrum while leaving the corresponding ion trap ranks unchanged. The number of matching ranks and within-one ranks between the ion trap and permuted triple quadrupole spectra was determined for each of the 10 000 random data sets and the distributions of values are plotted as histograms in Figure 3. The values observed in our experimental comparison are shown in red on the histogram as well. As shown in Figure 3a, the distribution of exact rank matches between ion trap and triple quadrupole ranks ranges from approximately 200 to 300 out of a potential of 1548 fragment ions, corresponding to 12.9% to 19.4% of the peaks. This is contrasted by the 738 exactly matched peaks in the experimental data set, corresponding to 48% of all fragment ions. Similarly, Figure 3b shows that the distribution of peaks with ion trap and triple quadrupole ranks within one rank of each other ranges from approximately 600 to 800 of 1548 peaks, corresponding to a relative range of 38.8% to 51.7%. This is contrasted by the 1276 peaks in the observed data set with within-one ranks, corresponding to 82% of all peaks. Since not one of the 10 000 randomly generated data sets yielded a larger proportion of matching ranks or within-one ranks, the analysis resulted in a *p*-value of 0 in both cases, indicating that the numbers of matching and nearly matching fragment ion ranks between instruments are statistically significant. This confirms that the rank order correlation between ion trap and triple quadrupole spectra, or at least the top-ranking y-type ions thereof, is highly significant.

Relative Intensities of y-Type Ions Are Strongly Correlated Across Ion Trap and Triple Quadrupole Platforms

To assess the correlation of the ion trap and triple quadrupole spectra in a quantitative manner, the relative intensities of the fragment ions of the two sets of spectra were compared by calculating the dot product between each normalized ion trap spectrum and corresponding triple quadrupole spectrum, creating a distribution of dot product values for the data set. Of these 258 dot products, over 70% assumed values of 0.95 or greater (Figure 4). Since normalized dot products range from zero to unity, with unity signifying an identical match, these high dot product values indicate a high degree of correlation between the relative intensities of the spectra derived from the two different instruments.

In order to test the statistical significance of this observed intensity correlation, we compared the observed dot product distribution to that of a data set with relative intensity randomized. This data set was generated by replacing the relative intensities of each triple quadrupole spectrum with those from a different, randomly selected spectrum from the data set, while leaving the ion trap spectra unchanged. This randomized data set yielded a relatively uniform dot product distribution, as expected for a probabilistic data set (Figure 5, Supplemental Figure 2). This is contrasted by the skewed distribution for the observed data (also in Figure 5, Supplemental Figure 2), the large majority of which are in the 0.9–1.0 range. A Wilcoxon rank-sum resulted in a *p*-value of 2.53×10^{-6} , confirming that the difference in dot product

distribution is highly statistically significant. These dot product distributions therefore demonstrate that the relative intensities of y-type ions from ion trap and triple quadrupole spectra are strongly correlated.

The method of randomization used for this relative intensity randomization test was one of many valid options. This method, in which the triple quadrupole spectrum for each peptide was randomized by replacing the relative intensity values for its constituent fragment ions with those from a randomly selected peptide from the data set, was chosen since it most closely preserved the dependence structure of the relative intensities of the spectra (i.e., by using real relative intensity values from other spectra). The preservation of this structure came at the expense of randomness, however, since the individual relative intensities were not completely random, but instead dependent upon the randomly selected peptide. This lack of true randomness is reflected in the dot product distribution for the random data set (Figure 5), which is not entirely uniform across all dot product values, but instead is slightly biased toward the upper half of the spectrum. The fact that the dot product distribution for the observed data set is still entirely distinct from the random distribution indicates that the strong correlation of ion trap and triple quadrupole spectra is not only distinct from a random correlation, but it is also distinct from the loose correlation of any two random spectra, which ultimately provides more support for the observed ion trap-triple quadrupole correlation. To supplement this analysis, the relative intensity randomization test was repeated with two other methods of randomization, both of which created random data sets that incorporated increasingly more randomness and correspondingly less preservation of the dependence structure of the spectra. In the first method (Supplemental Figure 3), each triple quadrupole spectrum was randomized by permuting the existing relative intensity values for the constituent fragment ions. In the second method (Supplemental Figure 4), each triple quadrupole spectrum was randomized by choosing random AUC values from the data set for the fragment ions from the given spectrum and renormalizing to the new top-ranking value to produce the randomized relative intensities. Both of these alternate methods produced random data sets with dot product distributions characterized by more uniformity than one included in the main analysis, making the distinction between the observed distribution and the random distribution all the more evident.

Ion Trap-Triple Quadrupole Correlation Expected for Instruments from All Vendors

The analysis presented here is based on a consensus spectral library built from thousands of spectra acquired by many different ion trap instruments and on MRM data acquired in our laboratory by a single triple quadrupole (Waters Quattro Premier). While only a single, well-optimized triple quadrupole instrument was used, there is no indication that the observed correlation between the y-ion spectra of the two mass spectrometers is specific to our particular instrument or its particular vendor. To the contrary, our results confirm a hypothesis rooted in theoretical and empirical knowledge of the physics of the two types of mass spectrometers, independent of any specific instrument or vendor, suggesting that our results should be instrument- and vendor-independent as well. We intend to verify this expectation with future instrumentation, but anticipate that the proteomics community will also seek confirmation via correlation analyses based on other well-calibrated ion trap and triple quadrupole instruments using a workflow similar to the one presented here.

Conclusion

Through the analysis of the rank orders and relative intensities of the top-ranking y-ion fragments of ion trap and triple quadrupole spectra from doubly charged peptides, we have demonstrated a strong correlation between these ions across platforms. While this analysis included only doubly charged peptides, we intend to conduct a similar analysis for triply charged peptides, once optimized instrument parameters are established for this class of

peptide, and we expect to find a similar y-ion correlation. The implication of the correlation found here is that any ion trap-discovered pair of precursor peptide and y-ion fragment can be directly targeted as an MRM transition on a triple quadrupole mass spectrometer, at least for doubly charged peptides. This finding supports the application of ion trap-derived consensus spectral libraries to the development of MRM assays for triple quadrupole-enabled experiments, permitting the investigator to mine the vast stores of shotgun MS data for direct use in MRM-MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Arnott D, Kishiyama A, Luis EA, Ludlum SG, Marsters JC Jr, Stults JT. Selective detection of membrane proteins without antibodies: a mass spectrometric version of the Western blot. *Mol. Cell. Proteomics* 2002;1(2):148–156. [PubMed: 12096133]
2. Anderson L, Hunter CL. Quantitative mass spectrometric multiple reaction monitoring assays for major plasma proteins. *Mol. Cell. Proteomics* 2006;5(4):573–588. [PubMed: 16332733]
3. Kostiainen R, Kotiaho T, Kuuranne T, Auriola S. Liquid chromatography/atmospheric pressure ionization-mass spectrometry in drug metabolism studies. *J. Mass Spectrom* 2003;38(4):357–372. [PubMed: 12717747]
4. Sannino A, Bolzoni L, Bandini M. Application of liquid chromatography with electrospray tandem mass spectrometry to the determination of a new generation of pesticides in processed fruits and vegetables. *J. Chromatogr., A* 2004;1036(2):161–169. [PubMed: 15146917]
5. Tai SS, Bunk DM, White Et, Welch MJ. Development and evaluation of a reference measurement procedure for the determination of total 3,3',5-triiodothyronine in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. *Anal. Chem* 2004;76(17):5092–5096. [PubMed: 15373447]
6. Lee MS, Kerns EH. LC/MS applications in drug development. *Mass Spectrom. Rev* 1999;18(3–4):187–279. [PubMed: 10568041]
7. Tiller PR, Cunniff J, Land AP, Schwartz J, Jardine I, Wakefield M, Lopez L, Newton JF, Burton RD, Folk BM, Buhrman DL, Price P, Wu D. Drug quantitation on a benchtop liquid chromatography-tandem mass spectrometry system. *J. Chromatogr., A* 1997;771(1–2):119–125. [PubMed: 9210315]
8. Wolf-Yadlin A, Hautaniemi S, Lauffenburger DA, White FM. Multiple reaction monitoring for robust quantitative proteomic analysis of cellular signaling networks. *Proc. Natl. Acad. Sci. U.S.A* 2007;104(14):5860–5865. [PubMed: 17389395]
9. Keshishian H, Addona T, Burgess M, Kuhn E, Carr SA. Quantitative, multiplexed assays for low abundance proteins in plasma by targeted mass spectrometry and stable isotope dilution. *Mol. Cell. Proteomics* 2007;6(12):2212–2229. [PubMed: 17939991]
10. Stahl-Zeng J, Lange V, Ossola R, Eckhardt K, Krek W, Aebersold R, Domon B. High sensitivity detection of plasma proteins by multiple reaction monitoring of N-glycosites. *Mol. Cell. Proteomics* 2007;6(10):1809–1817. [PubMed: 17644760]
11. Martin DB, Holzman T, May D, Peterson A, Eastham A, Eng J, McIntosh M. MRMer: An interactive open-source and cross-platform system for data extraction and visualization of multiple reaction monitoring experiments. *Mol. Cell. Proteomics* 2008;7(11):2270–2278. [PubMed: 18641041]
12. Desiere F, Deutsch EW, King NL, Nesvizhskii AI, Mallick P, Eng J, Chen S, Edes J, Loevenich SN, Aebersold R. The PeptideAtlas project. *Nucleic Acids Res* 2006;34(Database issue):D655–D658. [PubMed: 16381952]

13. Deutsch EW, Lam H, Aebersold R. PeptideAtlas: a resource for target selection for emerging targeted proteomics workflows. *EMBO Rep* 2008;9(5):429–434. [PubMed: 18451766]
14. Falkner JA, Falkner JW, Andrews PC. ProteomeCommons.org JAF: reference information and tools for proteomics. *Bioinformatics* 2006;22(5):632–633. [PubMed: 16434446]
15. Frewen BE, Merrihew GE, Wu CC, Noble WS, MacCoss MJ. Analysis of peptide MS/MS spectra from large-scale proteomics experiments using spectrum libraries. *Anal. Chem* 2006;78(16):5678–5684. [PubMed: 16906711]
16. Lam H, Deutsch EW, Eddes JS, Eng JK, King N, Stein SE, Aebersold R. Development and validation of a spectral library searching method for peptide identification from MS/MS. *Proteomics* 2007;7(5):655–667. [PubMed: 17295354]
17. Falkner JA, Falkner JW, Yocum AK, Andrews PC. A spectral clustering approach to MS/MS identification of post-translational modifications. *J. Proteome Res* 2008;7(11):4614–4622. [PubMed: 18800783]
18. Lam H, Deutsch EW, Eddes JS, Eng JK, Stein SE, Aebersold R. Building consensus spectral libraries for peptide identification in proteomics. *Nat. Methods* 2008;5(10):873–875. [PubMed: 18806791]
19. Paizs B, Suhai S. Fragmentation pathways of protonated peptides. *Mass Spectrom. Rev* 2005;24(4):508–548. [PubMed: 15389847]
20. Vachet RW, Ray KL, Glish GL. Origin of product ions in the MS/MS spectra of peptides in a quadrupole ion trap. *J. Am. Soc. Mass Spectrom* 1998;9(4):341–434. [PubMed: 9879364]
21. Jonscher KR, Yates JR III. The quadrupole ion trap mass spectrometer—a small solution to a big challenge. *Anal. Biochem* 1997;244(1):1–15. [PubMed: 9025900]
22. Hunt DF, Yates JR III, Shabanowitz J, Winston S, Hauer CR. Protein sequencing by tandem mass spectrometry. *Proc. Natl. Acad. Sci. U.S.A* 1986;83(17):6233–6237. [PubMed: 3462691]
23. Roepstorff P, Fohlman J. Proposal for a common nomenclature for sequence ions in mass spectra of peptides. *Biomed. Mass Spectrom* 1984;11(11):601. [PubMed: 6525415]
24. Biemann K. Appendix 5. Nomenclature for peptide fragment ions (positive ions). *Methods Enzymol* 1990;193:886–887. [PubMed: 2074849]
25. Dongre AR, Jones JL, Somogyi A, Wysocki VH. Influence of peptide composition, gas-phase basicity, and chemical modification on fragmentation efficiency: evidence for the mobile proton model. *J. Am. Chem. Soc* 1996;118(35):8365–8374.
26. Tang XJ, Boyd RK. An investigation of fragmentation mechanisms of doubly protonated tryptic peptides. *Rapid Commun. Mass Spectrom* 1992;6(11):651–657. [PubMed: 1467549]
27. Polce MJ, Ren D, Wesdemiotis C. Dissociation of the peptide bond in protonated peptides. *J. Mass Spectrom* 2000;35(12):1391–1398. [PubMed: 11180629]
28. Wysocki VH, Tsaprailis G, Smith LL, Brei LA. Mobile and localized protons: a framework for understanding peptide dissociation. *J. Mass Spectrom* 2000;35(12):1399–1406. [PubMed: 11180630]
29. Huang Y, Triscari JM, Tseng GC, Pasa-Tolic L, Lipton MS, Smith RD, Wysocki VH. Statistical characterization of the charge state and residue dependence of low-energy CID peptide dissociation patterns. *Anal. Chem* 2005;77(18):5800–5813. [PubMed: 16159109]
30. Kapp EA, Schutz F, Reid GE, Eddes JS, Moritz RL, O'Hair RA, Speed TP, Simpson RJ. Mining a tandem mass spectrometry database to determine the trends and global factors influencing peptide fragmentation. *Anal. Chem* 2003;75(22):6251–6264. [PubMed: 14616009]
31. Martin DB, Eng JK, Nesvizhskii AI, Gemmill A, Aebersold R. Investigation of neutral loss during collision-induced dissociation of peptide ions. *Anal. Chem* 2005;77(15):4870–4882. [PubMed: 16053300]
32. Martin DB, Holzman T, May D, Peterson A, Eastham A, Eng J, McIntosh M. MRMer, an interactive open source and cross-platform system for data extraction and visualization of multiple reaction monitoring experiments. *Mol. Cell. Proteomics* 2008;7(11):2270–2278. [PubMed: 18641041]
33. Smolka M, Zhou H, Aebersold R. Quantitative protein profiling using two-dimensional gel electrophoresis, isotope-coded affinity tag labeling, and mass spectrometry. *Mol. Cell. Proteomics* 2002;1(1):19–29. [PubMed: 12096137]

34. Bjellqvist B, Basse B, Olsen E, Celis JE. Reference points for comparisons of two-dimensional maps of proteins from different human cell types defined in a pH scale where isoelectric points correlate with polypeptide compositions. *Electrophoresis* 1994;15(3-4):529-539. [PubMed: 8055880]
35. Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez JC, Frutiger S, Hochstrasser D. The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. *Electrophoresis* 1993;14(10):1023-1031. [PubMed: 8125050]
36. Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, MR.; Appel, RD.; Bairoch, A. *The Proteomics Protocols Handbook*. Totowa, NJ: Humana Press; 2005.
37. Klimek J, Eddes JS, Hohmann L, Jackson J, Peterson A, Letarte S, Gafken PR, Katz JE, Mallick P, Lee H, Schmidt A, Ossola R, Eng JK, Aebersold R, Martin DB. The standard protein mix database: a diverse data set to assist in the production of improved peptide and protein identification software tools. *J. Proteome Res* 2008;7(1):96-103. [PubMed: 17711323]

Figure 1.

General workflow of the presented analysis: (1) generation of consensus spectral libraries from ion trap MS/MS data for the two sample types of interest; in this case, a yeast spectral library created by NIST was downloaded from PeptideAtlas.org and an 18 mix spectral library was generated in-house using SpectraST, based on our previously published and publicly available shotgun MS data; (2) creation of an MRM transition list for each of the three samples in the analysis, a low-*pI* yeast fraction, a high-*pI* yeast fraction, and 18 mix, from an organism-appropriate spectral library using the software tool MaRiMba; each yeast MRM list was restricted to peptides observed in discovery MS runs conducted prior to this analysis; (3) triple quadrupole-enabled MRM-MS targeting of the top six *y*-ion transitions of each peptide in the three samples; (4) quantification of the MRM data using MRMer to calculate the area under the curve (AUC) of each transition elution peak; and (5) comparison of the triple quadrupole-generated transition AUC values to their corresponding ion trap-derived spectral library peak intensities in order to assess the similarity of the spectra from the two instruments.

Figure 2. Agreement between peak ranks across platforms for (a) low-pI yeast, (b) high-pI yeast, (c) 18 mix, and (d) the combined data set. Ranks observed in the ion trap library spectra are compared with the ranks found experimentally on the triple quadrupole. A total of 1548 fragment peaks from 258 peptide spectra are included in the comparison (combined data set). Boxes along the diagonal represent matching ion trap and QQQ ranks, while the boxes directly above and below the diagonal represent a one-rank difference between instruments.

Figure 3. Comparison of rank order similarity between ion trap and triple quadrupole spectra for the 10 000 randomized data sets of the rank order permutation test (white bars) and the observed data set (red line, boxed number). This comparison is made for both (a) the number of peaks with matching ranks in both instruments and (b) the number of peaks with at most a one-rank difference between instruments.

Figure 4. Distribution of dot products between the normalized ion trap and triple quadrupole spectra for each peptide. The distribution for each individual data set is provided, as well as the distribution for the combined data set. All distributions illustrate the same trend: the dot products assume large values (>0.95) for the majority ($>70\%$) of peptides, signifying a strong correlation between the spectra from the two instruments.

Figure 5. The distribution of normalized dot products for the spectra in the observed data set (red line) and in the randomized data set (black line). The distinct distributions highlight the stark difference between the observed experimental data and randomness, indicating that the correlation between ion trap and triple quadrupole spectra is significant and nonrandom. Similar plots for the individual data sets are provided in Supplemental Figure 4.

Table 1

An Excerpt of the Tabulated Data on Which the Spectral Correlation Analyses Are Based^a

protein	peptide	prec m/z	prod m/z	fragment	sample	experimental-QQQ				library-ion trap			
						AUC	rank order	relative intensity	rank match	intensity	rank order	relative intensity	
YDR388W	GNVEEQTDALTTTHFK	901.95	532.30	y4	Low-pI yeast	498649	1	100.0	0	7843	2	78.4	
YDR388W	GNVEEQTDALTTTHFK	901.95	746.30	y6	Low-pI yeast	367296	2	73.7	0	10000	1	100.0	
YDR388W	GNVEEQTDALTTTHFK	901.95	1045.30	y9	Low-pI yeast	217902	3	43.7	0	5764	5	57.6	
YDR388W	GNVEEQTDALTTTHFK	901.95	1146.50	y10	Low-pI yeast	217631	4	43.6	0	7309	3	73.1	
YDR388W	GNVEEQTDALTTTHFK	901.95	930.50	y8	Low-pI yeast	187535	5	37.6	0	7176	4	71.8	
YDR388W	GNVEEQTDALTTTHFK	901.95	859.50	y7	Low-pI yeast	102327	6	20.5	1	4822	6	48.2	
YDL084W	TAVFYGGTPISK	620.83	659.37	y7	High-pI yeast	18063116	1	100.0	1	10000	1	100.0	
YDL084W	TAVFYGGTPISK	620.83	822.44	y8	High-pI yeast	15182154	2	84.1	1	8438	2	84.4	
YDL084W	TAVFYGGTPISK	620.83	969.51	y9	High-pI yeast	10944881	3	60.6	1	7734	3	77.3	
YDL084W	TAVFYGGTPISK	620.83	444.28	y4	High-pI yeast	8930639	4	49.4	1	5256	4	52.6	
YDL084W	TAVFYGGTPISK	620.83	1068.58	y10	High-pI yeast	2302069	5	12.7	1	2153	5	21.5	
YDL084W	TAVFYGGTPISK	620.83	545.33	y5	High-pI yeast	1008182	6	5.6	1	1216	6	12.2	
Q29443	NYELLC[160]GDNTR	677.81	722.29	y6	18 Mix	164326	1	100.0	1	10000	1	100.0	
Q29443	NYELLC[160]GDNTR	677.81	835.37	y7	18 Mix	135696	2	82.6	1	6119	2	61.2	
Q29443	NYELLC[160]GDNTR	677.81	562.26	y5	18 Mix	63110	3	38.4	1	5157	3	51.6	
Q29443	NYELLC[160]GDNTR	677.81	948.46	y8	18 Mix	41916	4	25.5	1	3704	4	37.0	
Q29443	NYELLC[160]GDNTR	677.81	505.23	y4	18 Mix	5325	5	3.2	0	946	6	9.5	
Q29443	NYELLC[160]GDNTR	677.81	1077.50	y9	18 Mix	3093	6	1.9	0	3392	5	33.9	

^aThis data includes the MRMer-calculated AUC value for each triple quadrupole-targeted transition and the corresponding spectral library-derived peak intensity. The resulting rank orders and relative intensities are also included, along with basic information about each transition (e.g., protein, peptide sequence, m/z values, etc.). The full data set, which includes all data from all three samples, is provided in Supplemental Table 2.