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Short communication

Improved mass analysis of intact proteins by ion trap instrument on a chromatographic time scale via data-dependant enhanced resolution scan

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ABSTRACT

Analyzing highly charged protein ions by ion trap instruments has been hindered by the low resolving power and the space charge effect. To improve mass resolution, the resonant ejection scan rate was often decreased, causing long cycle time that was not compatible with a chromatographic time scale. We described a new method that allowed the acquisition of high-resolution protein mass spectra on a chromatography time scale. The method was based on the data-dependant enhanced resolution scan (DDER) function of the triple quadrupole linear ion trap (Q Trap). We demonstrated the effectiveness of the method by analyzing liquid chromatography-resolved polypeptide components of a monoclonal antibody. The results showed that DDER-derived spectra significantly improved the resolution and accuracy for deconvoluted mass. Our approach would extend the utilities of ion trap instruments in protein analysis.

1. Introduction

Mass spectrometry has been extensively used to assign the molecular masses of protein molecules. Deconvolution of electrospray-ionized protein ions is a preferred technique for mass determination [1–3] for its high mass accuracy, the availability of deconvolution software, and the ease of coupling to LC for sample desalting. A variety of mass spectrometry instruments can be used to collect the mass spectra, including ion trap (IT), orthogonal time-of-flight (oTOF), quadrupole time-of-flight (Q-TOF), and Fourier transform ion cyclotron (FTICR) instruments. Analyzing multiply charged protein molecules on IT was challenging, mainly because of the "space charge" effect and the low resolution at full scan speed [4,5]. As a result, TOF and FTICR instruments have become the preferred instruments for accurate mass determination [6–8].

An approach to improve the resolution of IT is to decrease the resonant ejection scan rate [9–13]. High-resolution spectra for small-sized proteins (<20 kDa) have been achieved with this approach [14–16]. To avoid space charging, the trap fill has to be kept at a low value and multiple scans have to be combined to achieve good ion statistics. The resulting acquisition time often approached several minutes, not compatible with on-line analysis. In the present study, we developed a method that enabled high-resolution analysis of protein molecules on a chromatographic time scale. We applied the data-dependant enhanced resolution scan (DDER) function on a hybrid triple quadrupole/linear ion trap instrument (Q Trap) [17], in which only the top ions of the charge envelope were allowed into the ion trap and scanned at reduced rate. While similar approaches were routinely applied in proteomic experiments for peptide charge state determination, to our knowledge it has never been applied to the analysis of multiple charged protein ions.

2. Experiment

The monoclonal antibody was recombinantly produced from mammalian cell culture and purified on Protein A affinity chromatography. Mass spectra were acquired in positive mode on a triple quadrupole linear ion trap mass spectrometer (Q Trap, Applied Biosystems, Foster City, CA). The antibody was incubated with 10 mM dithiothreitol (DTT) at 37 °C for 15 min to reduce the interchain disulfides. Two micrograms of the reduced antibody was injected to a homebuilt LC system. The light chain and heavy chain were resolved on a reverse-phase PLRP-S column (Polymer Laboratories, Amherst, MA) with water/acetonitrile/0.1% trifluroacetate (TFA) mobile phases. The gradient applied was 30% water/TFA at time zero, 60% acetonitrile/TFA at 25 min, and 80% acetonitrile/TFA at 30 min. The column temperature was held at 55 °C. The HPLC eluent was coupled to the Q Trap via a Turbo V electrospray ion source. The spray voltage was 4000 V, the nebulizing gas was 15, the source temperature was 180 °C, and the declustering potential was 50 V. The DDER mass scan was comprised of a regular scan at a rate of 4000 u/s from 1000 to 1700 u, then a data-dependant enhanced resolution scan on the eight most intense ions that passed

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a user-defined intensity threshold. The quadrupole was operated in the RF mode in regular scan and in the low resolution mode in enhanced resolution scan. The trap fill target is 10^8 for the regular scan and 10^7 for enhanced resolution scan. The maximum trap fill time is 100 ms. Once an ion is selected, ions within ±8 u from the selected ion were excluded to prevent re-acquisition of the same isotope cluster. The spectra were combined across the chromatographic peaks. Mass deconvolution of the combined spectra was carried out with the Bioanalyst program. Baseline reduction and 20 iterations were performed for the deconvolution.

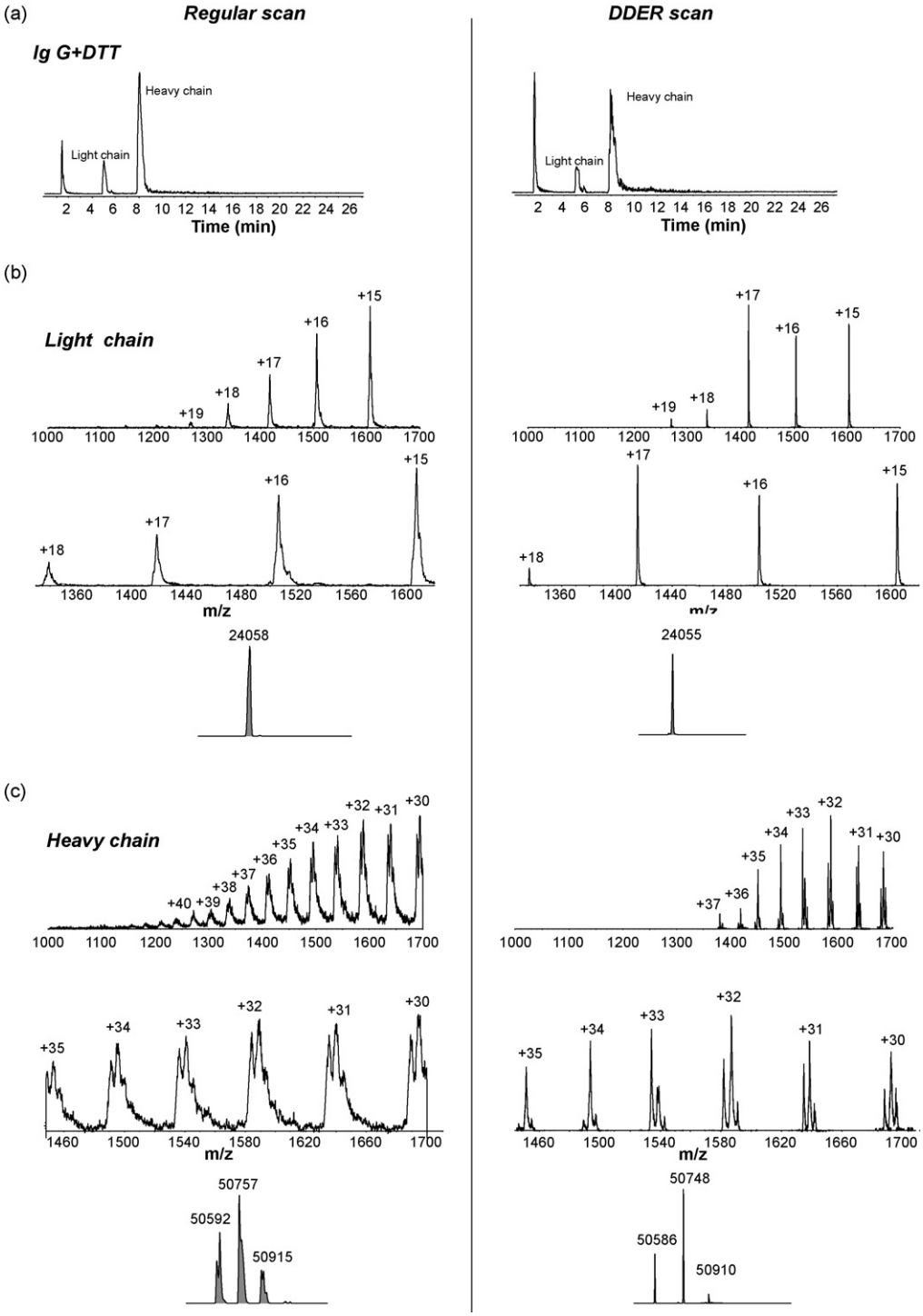

Fig. 1. Comparison of the regular scan and the DDER scan for IgG light chain and heavy chain analysis. (a) Total ion chromatography (TIC) of LC–MS analysis of the reduced IgG molecule. The IgG was reduced by DTT, and the light and heavy chains were separated by reverse-phase liquid chromatography. The column eluent was electrosprayed to a Q Trap mass spectrometry. The light chain was observed around 4 min and the heavy chain emerged at 8 min. (b) Mass spectra and deconvoluted mass of the IgG light chain. The full range of mass spectra (top pane) and the expanded view of peaks (middle pane) were compared, revealing sharper peaks and better signal-to-noise ratio for the DDER scan. The deconvoluted mass (lower pane) was also more accurate for the DDER scan. (c) Mass spectra and deconvoluted mass of the IgG heavy chain. The full range and expanded mass spectra were shown in the top and middle pane, respectively. The deconvoluted mass from the DDER mode was more accurate and the correct mass shift (162 u) between glycosylation isoforms ions was observed.

3. Results and discussion

We applied the DDER method to the characterization of a monoclonal antibody (or immunoglobulin, IgG). Upon DTT reduction, the IgG molecule released light chain and heavy chain, which were resolved on reverse-phase liquid chromatography and then electrosprayed to the Q Trap. The DDER method has three sequential steps: first, a survey scan at the full speed; second, the isolation of the eight most intense ions (20 u window surrounding the target mass); and third, a slow scan (250 u/s) across the isolation window. The resulted mass spectra were stitched together and deconvoluted to determine the molecular mass. In the regular scan mode, the quadrupole operates in the RF mode and the trap scans at a full speed (4000 u/s) with a trap fill target at 10^8 ions.

Fig. 1 compares the mass spectra recorded by the regular scan and the DDER scan. The total ion chromatogram (TIC) was shown in Fig. 1a: the light chain and heavy chain eluted around 4 and 8 min, respectively. While the two methods work via different mechanisms, no notable difference in chromatography profile was observed. The mass spectra across the chromatography peaks were generated for both the light and heavy chains in Fig. 1b and c, respectively. Only part of the charge state envelope was observed due to the limited mass range of the Q Trap (1700 u). The total scan time for DDER is very short (less than 20 s), compatible with on-line analysis. Notably, the DDER spectra of the light chain were sharper and has better signal-to-noise ratio than the regular scan.

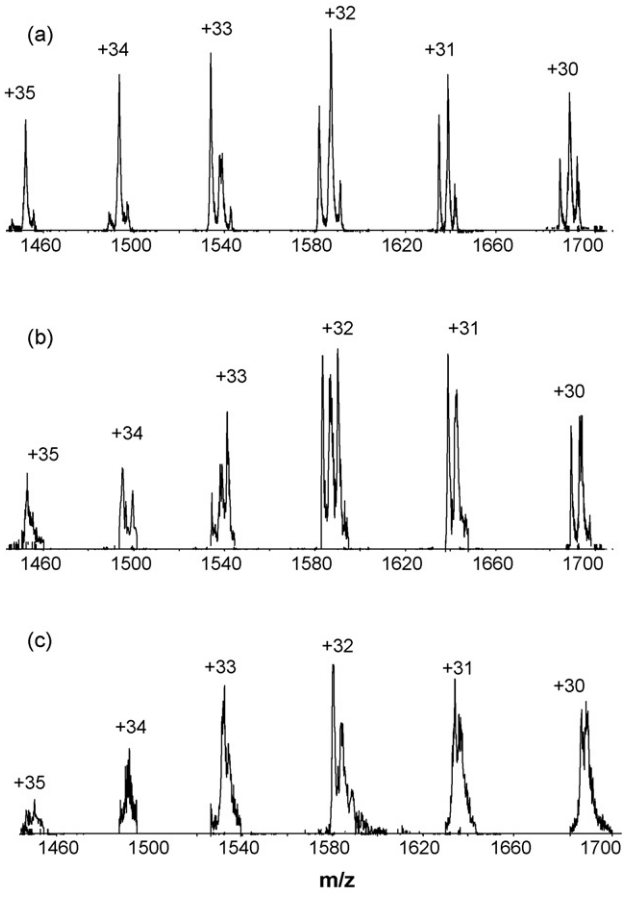

Fig. 2. The impact of scan speed and ion abundance on mass spectra resolution for multiply charged protein ions. The mass spectra for LC-resolved IgG heavy chain were collected using three conditions: (a) the DDER method (low trap fill and slow scan speed), (b) high trap fill and slow scan speed and (c) low trap fill and full scan speed. The results showed that the combination of low trap fill and slow scan speed generated the best signals.

The deconvoluted mass accuracy was also improved: 24,055 vs. 24,058 (24053, theoretical mass). The results demonstrated that by targeted ion isolation and scanning, the DDER method effectively reduced space charging and increased mass resolution.

The advantage of DDER method was further revealed in the heavy chain analysis (Fig. 1c), which was more challenging due to the heterogeneity introduced by N-linked glycosylation. The splitting pattern on the top of the peaks represents glycosylation isoforms that differs in one hexose moiety. The glycosylation isoforms were only partially resolved in the regular scan spectra, whereas baseline resolved in the DDER spectra. The deconvoluted mass is 50586, 50,748 and 50,910 for the DDER-derived spectra, close to the theoretical value (50,580, 50,742 and 50,904). The mass shift between adjacent glycosylation peaks is 162 u, consistent with a hexose moiety. The deconvoluted mass from the regular scan is less accurate (50,592, 50,757 and 50,915) and the mass shift deviated from 162 u (165 and 158). The results underscored the importance of high-resolution mass spectra for correct assignment of post-translational modifications.

We further dissected the individual contribution of reduced scan speed and low ion abundance to the improved mass resolution in DDER method, through varying one parameter while keeping the other constant. In Fig. 2, the mass spectra were collected for the IgG heavy chain in three modes: DDER mode (Fig. 2a); slow scan speed (250 u/s) at high trap fill (10^8) (Fig. 2b); and full scan speed (4000 u/s) at low trap fill (10^7) (Fig. 2c). Reducing the scan speed seemed to contribute more to the improved resolution, as the peaks in Fig. 2b were sharper than in Fig. 2c. The peaks in Fig. 2b resembled the DDER spectra in Fig. 2a at lower charge states (+30, +31 and +32), but at higher charge states (+33, +34 and +35) the peaks started to be affected by space charging and became broad. Hence, the combination of the two factors were required to deliver the optimal spectra quality.

4. Conclusion

The DDER method overcame some intrinsic limitations of IT for highly charged protein ions. The DDER method has two limitations: first, it is only applicable to purified proteins where only a single ion series or closely related ion series is present; and second, the stitched spectra are not quantitative. In addition to monoclonal antibody, the technique has been applied to a variety of protein molecules. Peak shape, signal-to-noise ratio and mass accuracy were significantly improved. The method could also be coupled with ion dissociation methods [18–20] for top-down protein analysis.

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