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Application Note 00691

Efficient Intact Protein Analysis Using a Hybrid Fourier Transform Mass Spectrometer

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Introduction

Novel insight into the character of large proteins or protein complexes can often be obtained by studying their high-resolution mass spectra. The ability to identify the degree and nature of post-translational modifications (PTM) or the ability to form a suggestion for a possible bonding pattern of an intact protein complex, provides us with better understanding of the structure and function of the protein. Also, modifications in a protein can have enormous consequences for an organism. Some lethal diseases, for example, are caused by protein modifications.

Performing analysis of large intact proteins on a Fourier Transform Mass Spectrometer (FTMS) is a demanding task. The difficulty is mainly due to the intrinsic effects, such as the space-charge effects or constructive/destructive interference of the FTMS. These effects can cause distortions in the collected transients, resulting in lower resolution and lower signal-to-noise ratio in the mass spectra. Fortunately, the current hybrid FTMS instruments provide an external ion isolation capability, which can be utilized to reduce the intrinsic effects and obtain higher resolution mass spectra for large intact proteins.

This application note describes an efficient method for analyzing large intact proteins with the Varian 901-MS QFT™ instrument. The external quadrupole region is used to isolate a single charge state of a large intact protein for excitation and detection. This results in higher resolution spectra and enhanced signal-to-noise ratio. An additional advantage is rapid acquisition speed; the high resolution is obtained acquiring a single mass spectrum while conventionally, the classical method requires signal averaging over several spectra. This method will be referred to as the improved method in the later discussions.

The described method is applied to study carbonic anhydrase. This is followed by an application to study an unidentified 36 kDa protein and intact bovine serum albumin (BSA). The mass spectrum of the +16 charge state of myoglobin is used to illustrate the data analysis performed with the PeakHunter™ software package.

Methods

All studied proteins were suspended in 50:50:2 water: methanol: acetic acid to a 1 µM concentration. The sample was injected 1 µL/min through an electrospray ionization source. The carbonic anhydrase II (Sigma C3934) and intact bovine serum albumin (Sigma A0281) were used as received. The 36 kDa protein was desalted using an LC system prior to the direct infusion studies.

All the spectra were collected with the 7 T Varian 901-MS QFT instrument in a broadband mode. The schematic of this hybrid instrument is shown in Figure 1. The first quadrupole region, labeled as Q1, was applied to perform the external ion isolation. The ions within the specified *m/z* range to be passed through were accumulated in the hexapole immediately following the second quadrupole region. The ion accumulation hexapole is labeled as Q3 in Figure 1. An 8 MWord transient digitizer and four-second transient time were applied in order to obtain high resolution. The external ion isolation step further increases the efficiency of the excitation and the detection of the ion in the ICR cell.

In order to perform a comparison of the conventional and the improved method, each of the studied proteins were also subjected to a more classical analysis. A spectrum of a series of charge states was obtained and signal averaging was applied for improved resolution and signal-to-noise ratio.

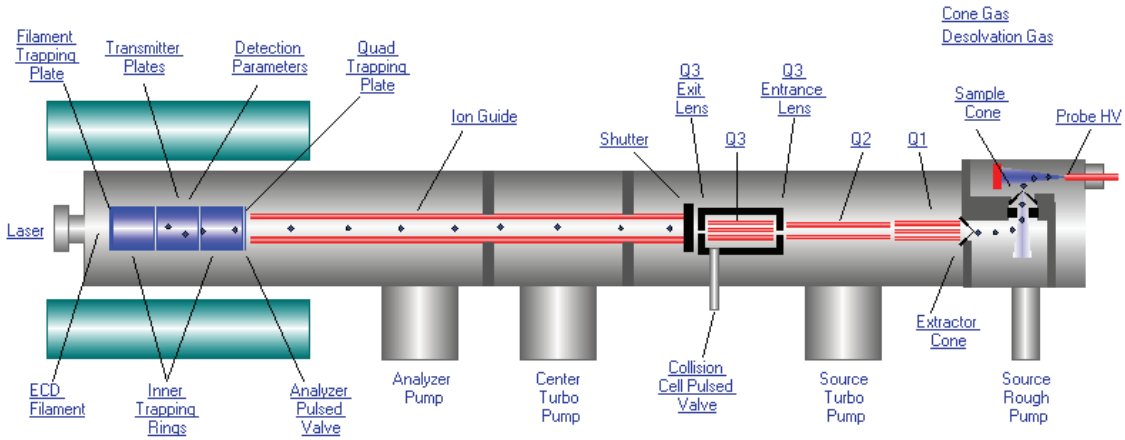

Figure 1 The schematics of the Varian 901-MS QFT instrument.

Discussion

Figure 2 shows a conventional mass spectrum of carbonic anhydrase. Fifty scans were collected in the classical analysis mode. The scans were signal averaged to obtain isotopically resolved envelopes and an approximate resolution of 91,000 for each of the charge states present in the spectrum.

Applying the improved method, the approximate resolution of 91,000 for carbonic anhydrase can be obtained with a single scan. This is shown in Figure 3. Comparing the close-ups of Figures 2 and 3, it can be seen that the improved method is very comparable to the classical method. Furthermore, the improved method has the advantage of an acquisition time of approximately 2% of that of the conventional one. Acquisition times are shorter because high resolution is obtainable with fewer scans per spectrum.

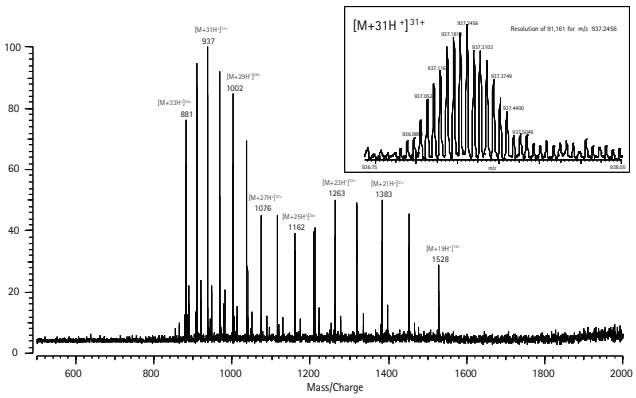

Figure 2 A mass spectrum of carbonic anhydrase. 50 scans were signal averaged to obtain an approximate 91,000 resolution and isotopically resolved envelopes for each charge-state.

Similar comparison is performed on an approximately 36 kDa protein, the ID of which is confidential. Figure 4 shows a high-resolution mass spectrum obtained with the conventional method. Here, 25 scans are collected to resolve the isotopic envelopes. Figure 5 illustrates that applying the improved method, the same results are obtained by acquiring a single scan. The isolation spectrum of the +30 charge state of the protein is shown as an example.

Figure 6 shows the application of the improved method to study the +47 charge state of intact bovine serum albumin (BSA). It can again be seen that applying the external ion isolation prior to the ion accumulation, the charge state is isotopically resolved by acquiring a single scan.

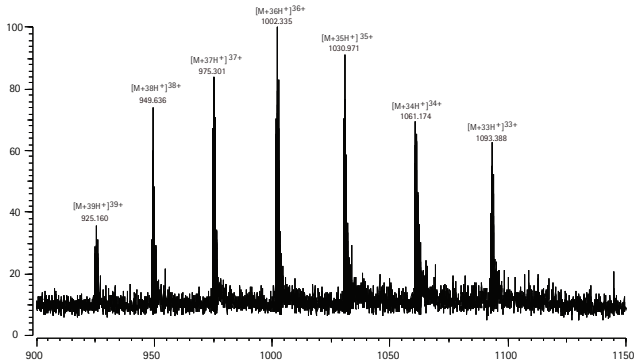

Figure 4 A mass spectrum of a 36 kDa protein (ID confidential). The conventional method of acquiring and signal averaging 25 scans was applied.

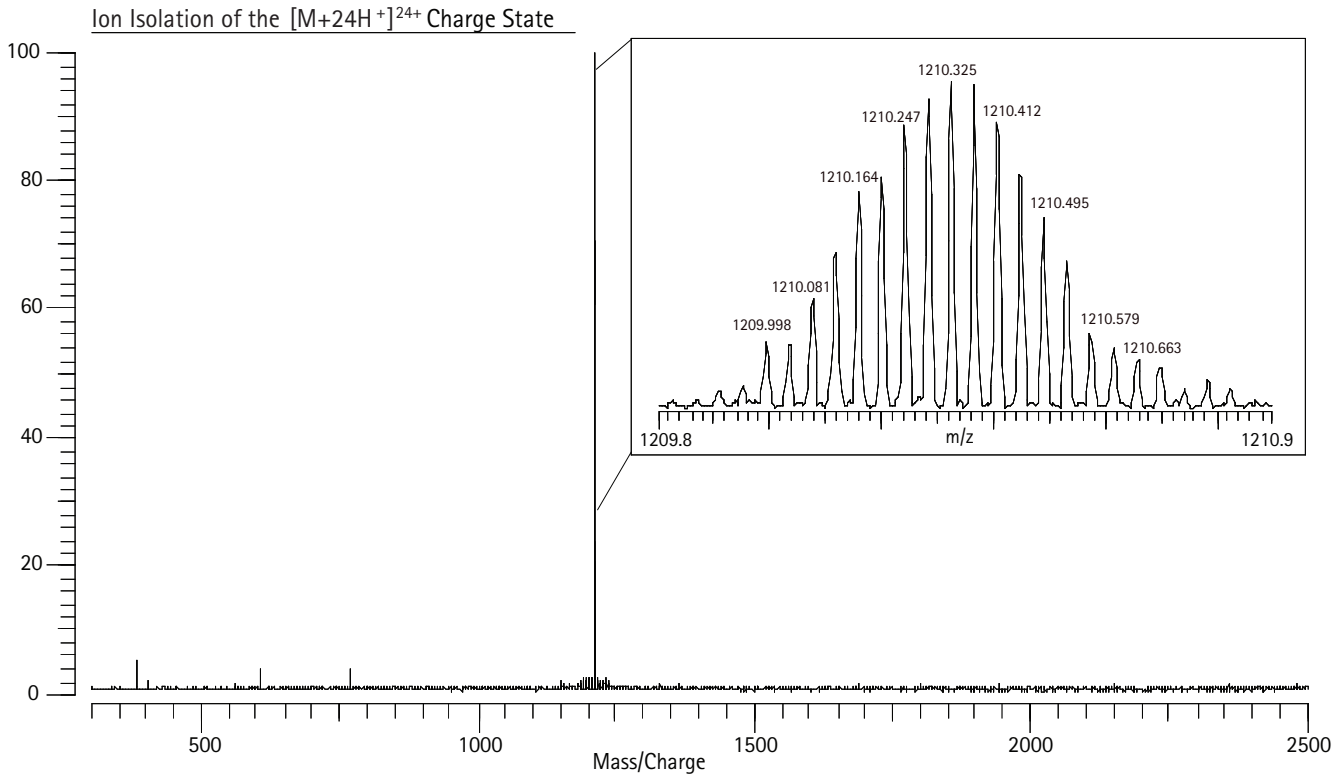

Figure 3 The improved method applied to carbonic anhydrase. Resolution of 91,000 for the isotopically resolved charge state was obtained with a single scan.

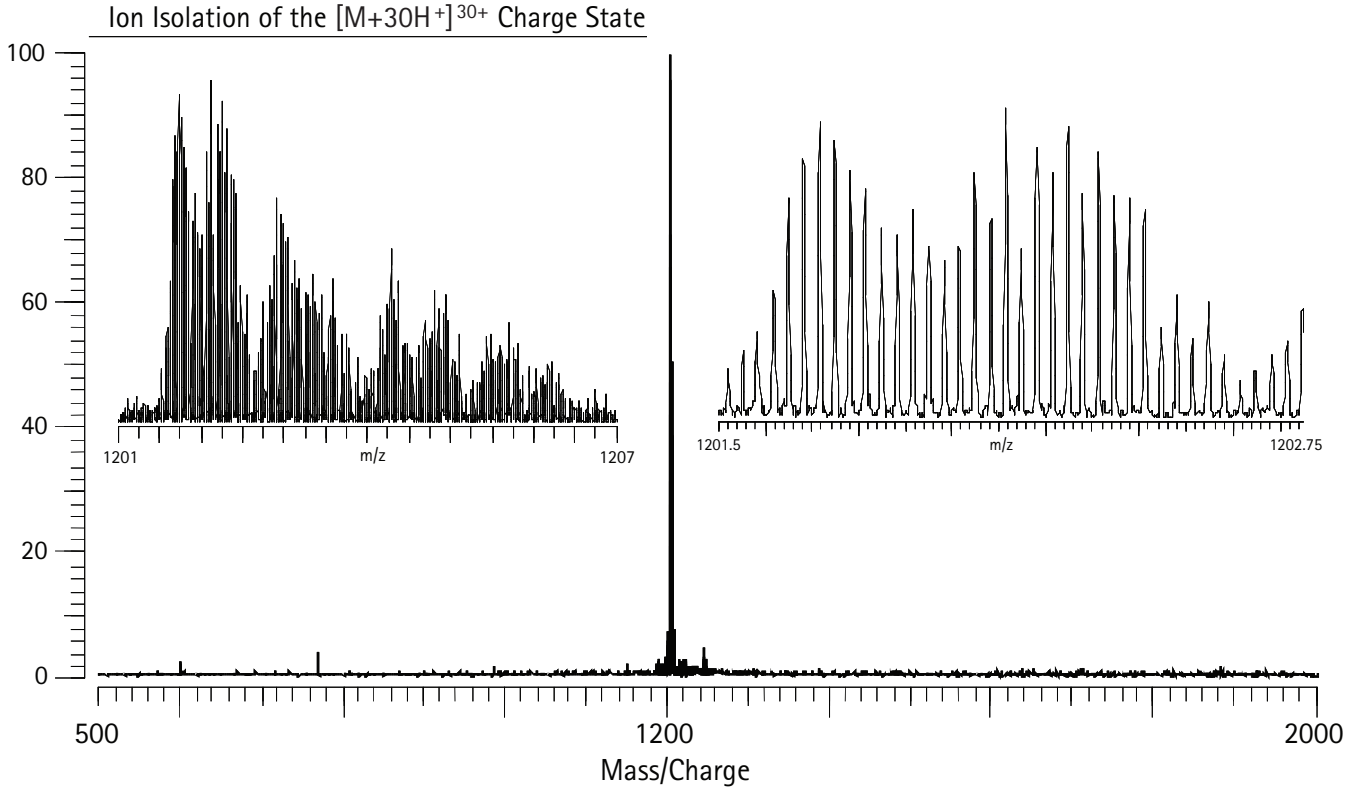

Figure 5 The 36 kDa protein analyzed applying a single scan of the improved method. The close-ups illustrate the isotopic resolution of the charge-state envelope.

All the acquired data can be analyzed with the PeakHunter™ software. A screenshot of the software is shown in Figure 7. Analysis of intact myoglobin using the discussed improved method is shown as an illustrative example. In Figure 7, the top row shows the acquired spectrum while the middle view illustrates the acquired data as a stick plot. In this mode the acquired data can be conveniently compared to the calculated data, which is shown in the bottom view. The detected peaks are grouped as isotopically resolved envelopes and listed as isotopic clusters on the left view as seen in Figure 7. Other features of PeakHunter include the efficient protein assignment or data export when desired.

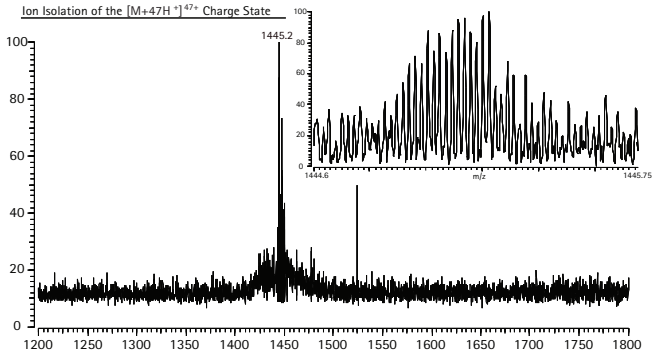

Figure 6 A mass spectrum of BSA acquired applying the improved method. A single scan was acquired to resolve the isotopic peaks.

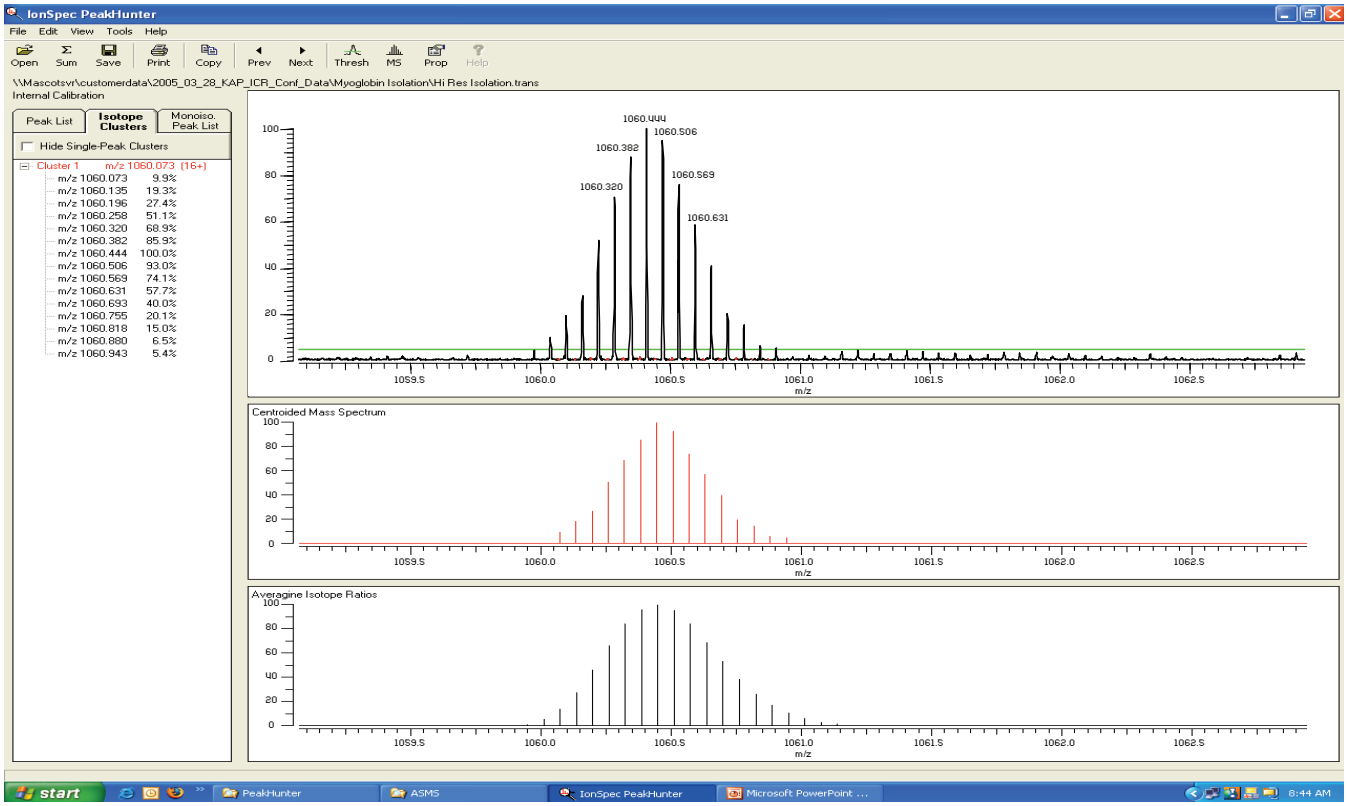

Figure 7 A screenshot of PeakHunter™ software showing an analysis of myoglobin acquired with the discussed improved method.

Conclusions

An efficient method of obtaining high-resolution mass spectra for intact proteins was discussed. This method utilizes the external ion isolation capability of the hybrid FTMS instruments. It was shown that the intrinsic effects that cause distortions in the transient can be reduced by applying the external ion isolation. With the improved method, higher resolution and enhanced signal-to-noise ratio are obtained with a fraction of the analysis time when compared to the conventional method of signal averaging.

This improved method also allows for more efficient fragmentation of the species of interest. Top-down sequencing can be performed with any of the in-cell fragmentation techniques in order to, for example, locate the post-translational modification sites of the protein.

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