International Journal of Mass Spectrometry 306 (2011) 227–231

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Towards data acquisition throughput increase in Fourier transform mass spectrometry of proteins using double frequency measurements

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ARTICLE INFO

Article history:
Received 6 September 2010
Received in revised form 8 November 2010
Accepted 29 November 2010
Available online 10 December 2010

Keywords:
Electrospray
ESI
Mass spectrometry
MS
Fourier transform mass spectrometry
FTMS
Ion cyclotron resonance mass spectrometry
ICR
Multiple frequency detection

ABSTRACT

Combined with high and ultra high pressure liquid chromatography, mass spectrometry-based peptide and protein structure analysis requires high resolution and mass accuracy achievable within short ion detection time. Although Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) offers highest resolution and mass accuracy among all types of mass spectrometers, it is unacceptably slow for many applications in proteomics requiring on-line sample pre-fractionation. Multiple frequency detection promises to increase the speed of ion detection in FT-ICR MS without the loss of resolving power, but its application to peptide and, especially, protein analysis has been negligible. In this report, we show that double frequency detection in high field FT-ICR MS indeed allows faster acquisition of high resolution mass spectra of proteins.

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1. Introduction

Resolving power increase in Fourier transform mass spectrometry (FTMS) remains to be a hot topic of the allied method and technique development [1–3]. Higher resolution in a given detection period directly translates into shorter experiment times at constant resolution. That is a necessary improvement for the increased performance of FTMS hyphenation with on-line liquid chromatography separation. In FT ion cyclotron resonance (FT-ICR) mass spectrometry the resolution and resolving power linearly increase with a frequency of ion cyclotron motion [4]. Therefore, ultrahigh resolution FT-ICR mass spectrometers require powerful superconducting magnets. Currently commercially available FT-ICR MS systems could be equipped with 12, 15 or 18 T magnets. However, magnets of that strength with wide bore size and sufficient magnetic field homogeneity are not only substantially more expensive and bulkier than the more common 7 and 9.4 T ones, but they also require additional method development and optimization for ion transfer through the magnetic field gradient and ion excitation to higher ion kinetic energy values [5]. Therefore, development of technologies that would allow achieving higher performance characteristics, e.g., increased resolving power for a given period of detection time, without going for a stronger magnet, is highly desirable.

Multiple frequency detection has long being realized as a method of choice to achieve increased resolving power in FT-ICR MS [6,7]. The idea of multiple frequency detection is to create 2 or more periods of induced current oscillation for only one period of ion cyclotron motion by increasing the number of detection plates. As a result, the frequency spectra are shifted into the higher frequency region which provides the corresponding increase of resolving power within the same time period [8–12]. Using multiple frequency detection scheme and multi-electrode cylindrical ICR cell configuration a record resolving power of 211,000,000 has been achieved for $^{132}Xe^{+}$ ions using 7 T FT-ICR MS instrument [13].

Although multiple frequency detection is a promising technique to improve the performance of the FT-ICR MS technique for peptide and protein analysis, the application of the method for protein ion detection has not been demonstrated. Whereas the ICR detection speed increase, especially with the nowadays common front-end hyphenation of ICR mass analyzer with fast linear and single quadrupole ion traps, would be very timely and important, the ideal scheme to be ready for implementation in a commercial system still has to be determined. Without this improvement, FT-ICR MS starts loosing the dominance in top down mass spectrometry

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doi:10.1016/j.ijms.2010.11.020

to the rapidly developing time-of-flight mass spectrometry-based and Orbitrap FTMS technology [14–17]. An alternative scheme for multiple, specifically double, frequency detection without ICR cell modification has been recently proposed and verified for peptide ion detection using lower magnetic fields of 1–5 T [9,18]. The reported preliminary results not only demonstrate the expected increase in resolving power, but also indicate the potential drawbacks of the method. Here, we extend the method application to protein ion detection and describe its first proof-of-principle implementation on FT-ICR MS build around a 12 T superconducting magnet.

2. Experimental

2.1. Sample preparation

Bovine cytochrome C protein (product number C2037) and Thermo calibration mixture (product number MSCAL5) were obtained from Sigma–Aldrich (Sigma–Aldrich, Buchs, Switzerland) and used without further purification. Bovine cytochrome C protein was dissolved in water and acetonitrile (50:49.5, v/v) with addition of 0.5% formic acid to a final concentration of ~10 μM.

2.2. Mass spectrometry

The experiments were performed on a hybrid LTQ FT-ICR MS (Thermo Scientific, Bremen, Germany) equipped with a 12 T superconducting magnet (Oxford Nanoscience, Oxford, UK). Magnet room-temperature bore diameter was ~100 mm and magnetic field homogeneity was better than 10 ppm inside of the ~50 mm diameter cylindrical ICR cell (ICR ultra cell from Thermo Scientific). Samples were ionized with a standard electrospray ion source (Thermo Scientific) at a flow rate of ~5 μl/min. Multiply protonated proteins were isolated (isolation width 10 m/z) and accumulated (AGC setting of 200,000 charges) in the linear quadrupole ion trap prior to further ion transfer into the ICR cell for analysis. Ion excitation was performed with the excitation amplitude set to the maximum allowed value (1.0 value for excitation amplitude in LTQ Tune advanced calibration menu). For each acquisition, the sum of 100 raw transients was acquired and further processed by MIDAS software using Hanning apodization and zero filling [19].

2.3. Double frequency measurements

To double the detection frequency, ion motion was detected twice per turn and per detection channel of the pre-amplifier, in contrast to the typical FT-ICR MS operation with single ion detection per turn. Mass spectra in both, single and double frequency operation modes were externally calibrated using the standard calibration solution (Thermo Scientific). The straightforward implementation of double frequency measurement experiment is shown in Fig. 1, left. Briefly, both detection plates were connected to a single input of the detection differential pre-amplifier, whereas the second pre-amplifier input was grounded [18]. In a typical FT-ICR MS operation mode, induced current signals coming from the two ICR cell detection electrodes are subtracted in the pre-amplifier, Fig. 1 right. Whereas in the double frequency operation mode, the 0 V signal (ground) is subtracted from the sum of both signals at the differential pre-amplifier, Fig. 1 left. Note that the scheme for the double frequency detection used in this work is different from the use of four ICR trap electrodes (pairs of excitation and detection plates) proposed earlier [6]. However, it allows switching of the instrument operation modes between the "conventional" and the double frequency ones without vacuum breaking followed by ICR trap re-design and re-wiring or using noise inducing electronic switches.

3. Results and discussion

The FT-ICR MS operation modes shown in Fig. 1 influence the amplitude modulation of the induced current in the corresponding way, as illustrated by the differently shaped transient signals of voltage (or charge) in the time domain, Fig. 2. Transient signals, acquired with the double frequency detection scheme for cytochrome C $[M+15H]^{15+}$ ions, Fig. 2a, exhibit a mixture of a signal detected at a double frequency, Fig. 2c, and a signal detected at a single "cyclotron" frequency, Fig. 2d. The shape of a latter transient clearly shows a sum of single and double frequency signals. The more intense signal packets contain both harmonics, whereas the minor signal packets contain only the second one. The conventional FT-ICR MS transient signal, Fig. 2b, shows the typical pattern for the multiply charged protein detection at a single frequency, Fig. 2e.

The presence of two frequencies in the transient signal acquired with double frequency set-up can be explained in the following way. The induced current signal from one of the detection electrodes in a frequency domain representation contains first, second, and higher harmonics:

$$\text{signal}_1 = a_1 \sin(\omega t) + a_2 \cos(2\omega t) + \cdots$$

For the second detection electrode, the induced current signal of each harmonic is shifted, by π and 2π relative to the first harmonic, ω, and the second harmonic, 2ω, respectively:

$$\text{signal}_2 = -a'_1 \sin(\omega t) + a'_2 \cos(2\omega t) + \cdots$$

For a typical FT-ICR MS operation, Fig. 1 right, these two signals are subtracted from each other at the differential pre-amplifier. However, in case of FT-ICR MS operating as depicted in Fig. 1 left, the resulting signal is a summation of the induced current signals from both detection electrodes:

$$\text{signal} = (a_1 - a'_1)\sin(\omega t) + (a_2 + a'_2)\cos(2\omega t) + \cdots$$

Therefore the presence of both frequencies in the transient signal in Fig. 2a is, presumably, due to the difference in signal amplitudes received from two detection electrodes that could be produced by misalignment of the center of the ion cloud motion and the ICR cell center, imperfections in the magnetic field orientation and in the ICR cell design. In a separate experiment, we demonstrated that the additional delay introduced prior to ion excitation modulates the ratio between the harmonics (data not shown).

Fig. 3 shows the FT-ICR mass spectra of cytochrome C $[M+15H]^{15+}$ ions acquired with the experimental set-up depicted in Fig. 1 left and corresponding transient signal shown in Fig. 2a. The considered induced current detection periods are 96, 192, 385, and 768 ms. Following the above mentioned presence of two frequencies in the transient signal, there are two major peaks in the mass spectrum. The first peak at $m/z = 816$ corresponds to the ion signal measured twice per period of ion rotation in the ICR cell and the second one at $m/z = 1632$ corresponds to the ion signal measured only once per period. Mass spectra were calibrated in the double frequency mode of operation. For 96 ms detection period only the double frequency operation mode could resolve the isotopic distribution of the 15+ charge state of cytochrome C, Fig. 3 bottom. Ion detection at longer periods resolves isotopic clusters of cytochrome C ions for both, single and double frequency detection modes. The isotopic clusters shown on the insets demonstrate the isotopic distributions comparable to those typically obtained in the conventional FT-ICR MS operation mode in terms of the number and relative amplitudes of the isotopic peaks in the cluster. Importantly, and as expected, the resolving powers for the second

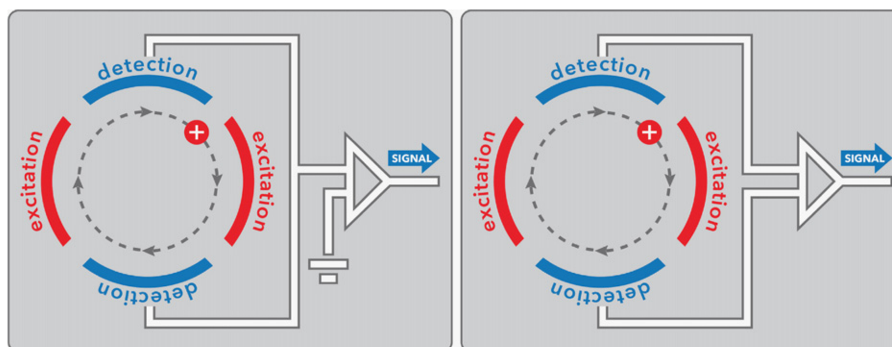

Fig. 1. Schematic drawing of the detection electrodes connection to the pre-amplifier in FT-ICR MS operated with (left) double and (right) single frequency ion detection, the latter one being the conventional FT-ICR MS operation mode.

harmonics are about 2 times higher than for the first harmonics, Fig. 3. Although present, higher harmonics were not recorded due to the substantial increase in the number of data points for low m/z range and the corresponding file size for long acquisition times.

Despite a two-fold increase in resolving power for the same transient duration, the experimental scheme, drawn in Fig. 1 left, reduces the signal to noise ratio compared to the conventional FT-ICR MS operation mode, Fig. 1 right. The additional noise sources are, presumably, the non-ideal contact grounding of the second pre-amplified input, as well as the fact that in the above equations the a_2 coefficient is always lower then a_1, and the ratio a_2/a_1 is increasing from 0 when ions are close to the ICR cell center to about 0.8 at the maximum ion cyclotron motion orbit. For estimation of the maximum harmonics ratio, the fast Fourier transform of the periodic pulsed function (1/4 of the period the function value is −1, the rest of the period −0) has been performed in MATLAB. Therefore, the signal to noise ratio for the double frequency operation mode is always lower than for the conventional FT-ICR MS operation. To increase the signal of the second harmonic, ions would need to be excited to the higher orbits. However, the coherent motion of ion clouds upon excitation to higher orbits suffers from the imperfections in excitation and trapping potentials due to increased amplitude of ions' axial oscillations [20]. Another important analytical characteristic of FT-ICR MS performance that may decay from operation in a double frequency detection mode is mass accuracy. Although it has been shown that mass accuracy is not significantly affected for peptide detection with double frequency at low magnetic fields [18], a detailed investigation is needed for protein analysis even at high magnetic fields. Here we report the mass accuracy for internal calibration of the standard Thermo calibration mixture and FT-ICR MS acquisition of a 768 ms transient, Table 1. The corresponding mass spectrum was calibrated for six peaks with a standard calibration equation accounting for the space charge effects [21]. The obtained RMS error of ∼0.058 ppm for this internal calibration confirms high accuracy of the enhanced second harmonic detection in 12 T FTMS. The application of the generated calibration coef-

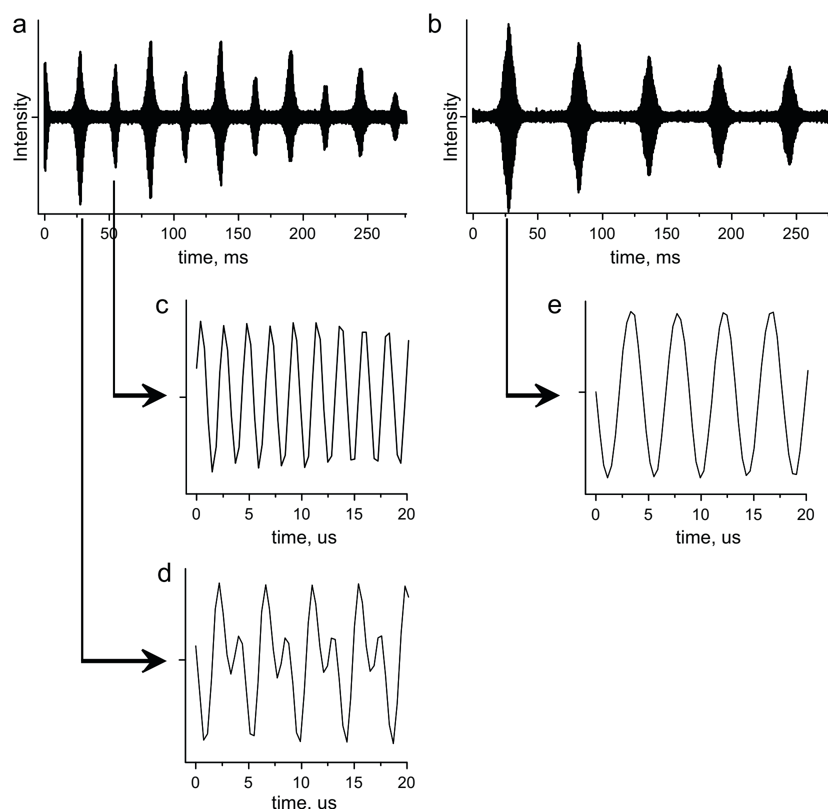

Fig. 2. Time-domain transient signals recorded from cytochrome C $[M+15H]^{15+}$ ions for the (a) double and (b) single frequency detection experimental configurations with the 12 T FT-ICR MS. The insets show the expanded segments of the time-domain transients.

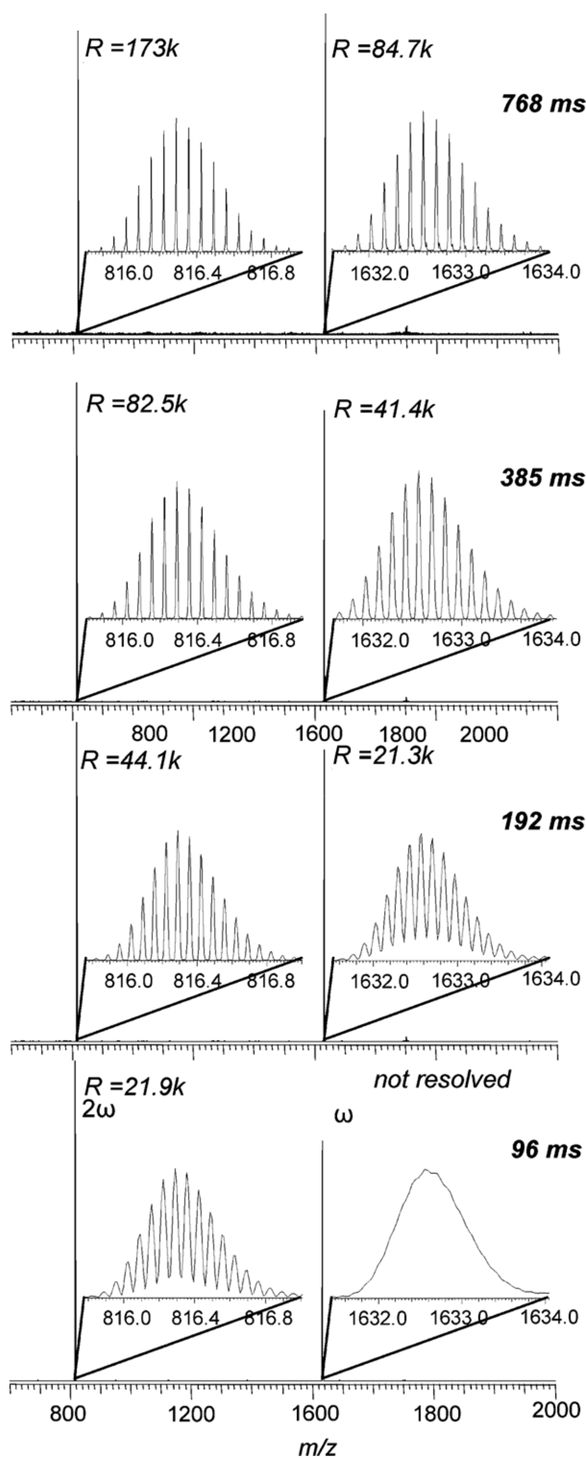

Fig. 3. FT-ICR MS of cytochrome C $[M+15H]^{15+}$ acquired at 12 T magnetic field and operated in double frequency measurement mode. The detection period was 96, 192, 385, 768, and 1536 ms, respectively, and the corresponding resolving power values are assigned to the isotopic clusters for both, single and double frequency detection modes. Mass spectra were calibrated in the double frequency mode of operation.

ficients for the external calibration of the mass spectra in Fig. 2 gives an average mass accuracy of ~2.5 ppm, which correlates with the typically received values for protein mass spectrometry on a given 12 T FT-ICR MS operated in a single frequency detection mode and externally calibrated mass spectra. We shall mention that the high mass accuracy values achieved after internal calibration and reported in Table 1 are 2–10 times better than the typically obtained ones in bottom-up proteomics with internal calibration. Whereas protein mass accuracy obtained with external calibration should be further, 2–3 fold, improved with a better calibration procedure.

Table 1
Internal mass calibration results for a standard calibration mixture (Thermo Scientific) performed using double frequency detection on a 12 T FT-ICR MS.

Frequency (Hz)	Theoretical m/z (Th)	Measured m/z (Th)	Error (ppm)
328,399.6732	1121.99702	1121.99692	−0.086
301,526.9436	1221.99064	1221.99076	0.099
278,719.5525	1321.98425	1321.98424	−0.008
259,119.7631	1421.97786	1421.97788	0.016
242,095.39	1521.97148	1521.97152	0.026
227,170.131	1621.96509	1621.96501	−0.047

Finally, the presence of the single frequency signals in the mass spectra imposes the constraints on the m/z range available for the non-overlapping mass measurements. In the typical bottom-up proteomics experiment the m/z region concerned is primarily between 400 and 900 m/z, which would correspond to 200–450 m/z region in a double frequency operation mode. Therefore, the overlap would be minor. In the typical top-down proteomics experiment the selected multiply charged protein ion also belongs to the same region. Upon fragmentation in the gas phase, product ions primarily cover the ±200–300 Da region around the isolated precursor ion m/z value. Therefore, precursor ion selection in top down mass spectrometry can be done with taking into account the m/z region concerned.

4. Conclusions

We demonstrated that double frequency detection in FT-ICR MS allows resolving the isotopic distribution of multiply charged protein ions as much as twice faster compared with “conventional” detection at the cyclotron frequencies. Therefore, same resolving power can be obtained within a twice shorter acquisition period that can be a promising feature of FT-ICR MS application in top down proteomics of intact proteins and large protein fragments. Additionally, the use of shorter acquisition times reduces the inhomogeneous line broadening due to frequency drifts during prolong detection as well as results in better quantitative assessment of the relative ion abundances from the peak intensity comparison. Nevertheless, the implemented double frequency detection set-up could be considered only as a proof-of-principle experiment. The implementation of double, or higher, frequency ion detection for protein analysis in FT-ICR MS with the true multi-electrode ICR cell has long being debated and attempted but not yet characterized in detail. We believe the current results should further promote multiple frequency detection in FT-ICR MS and trigger the necessary technique development and commercial implementation.

Acknowledgments

We thank Anton Kozhinov, Hisham Ben Hamidane, and Sasa Miladinovic for fruitful discussions and help in experiment preparation. This work was supported by the Swiss National Science Foundation (SNF project 200021-125147/1) and the Joint Russia-Switzerland Research Program (grant agreement 128357). MVG also thanks Russian Foundation for Basic Research (RFBR projects 08-04-01339 and 09-08-00633).

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