Dual track time-of-flight mass spectrometry for peptide quantification with matrix-assisted laser desorption/ionization

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RATIONAL: Previously, we reported a method (Anal. Chem. 2012, 84, 10332) for peptide quantification based on matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS). In the method, the peptide-to-matrix ion abundance ratio was utilized. Implementation of the method with a commercial MALDI-TOF can be somewhat inconvenient because matrix-derived ions are routinely deflected away to avoid detector saturation. A solution for this inconvenience is required.

METHODS: We installed a detector to acquire the TOF spectrum of the ions thrown away to avoid detector saturation. By sending the matrix- and peptide-derived ions along two different tracks and detecting them with different detectors, the inconvenience mentioned above could be avoided.

RESULTS: Excellent linearity of the calibration curves obtained by the dual track TOF spectrometry is demonstrated. The method also allows for the acquisition of the tandem mass spectrum of a selected peptide, which can be useful for its identification.

CONCLUSIONS: We devised the dual track MALDI-TOF MS method to avoid detector saturation and demonstrated that the quantification and identification of peptides can be performed simultaneously. Copyright © 2014 John Wiley & Sons, Ltd.

There is an increasing demand for the quantification of proteins present in tiny amounts.1,2 Mass spectrometry (MS) is one of the most promising techniques to meet this demand.3–5 Here, peptides generated by the enzymatic digestion of a protein, rather than the protein itself, are quantified.4 At the moment, probably the most powerful MS-based quantification method is to adopt peptides labeled with stable isotopes as internal standards5 and to use liquid chromatography (LC)/electrospray ionization (ESI)-MS.6 Alternatively, a peptide-internal standard mixture may be partially purified by two-dimensional (2D) gel electrophoresis and analyzed by matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS.7 However, both methods are costly and time-consuming and hence are not regarded as routine analytical tools.

In our recent investigation of the spectral variation in MALDI of peptides,8–10 we identified three factors affecting a MALDI spectrum: sample homogeneity, the peptide concentration (c(P)), and the effective temperature in the early matrix plume (Tearly).11,12 Here, c(P) is the peptide-to-matrix ion abundance ratio in the sample. Specifically, for homogeneous solid samples with a given c(P), we found that not only their MALDI spectral patterns, but also the absolute ion abundances (intensities) became similar regardless of the experimental condition when the spectra associated with the same Tearly were selected.9,10 By comparing the spectra acquired at the same Tearly for samples with different c(P) values, we found that the matrix-to-peptide proton transfer reaction could be treated as if it had been in equilibrium.10 This resulted in the following relationship between the peptide-to-matrix ion abundance ratio in the spectrum and the peptide concentration in the sample.

\[ I([P + H]^+)/I([M + H]^+) \propto c([P]) \] (1)

Here, I denotes the abundance of each ion in the plume. We demonstrated that this simple proportionality relationship could be used for the quantification of a peptide(s) present in a complex mixture.12,13 It should be noted that the quantification method based on the linear I([P + H]^+)/I([M + H]^+) vs. c([P]) plot is expected to be quick and inexpensive compared to the method utilizing peptides labeled with several stable isotopes as internal standards.5,7,13–15 We also found that the calibration curve for a peptide determined with a particular instrument remained virtually the same even several months after its determination (unpublished data). Therefore, a peptide can be quantified once its calibration curve is available from a previous measurement. This is in contrast to internal standard based quantification methods, which require the addition of the internal standard for each analyte to each sample.

A characteristic of the quantification method based on the I([P + H]^+)/I([M + H]^+) vs. c(P) plot is that not only the abundance of the peptide ion, but also that of the matrix

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ion must be measured.\textsuperscript{[12]} Although the measurement of the abundance of a matrix ion by MALDI-TOF is not difficult at all, this is not routinely done in a spectral acquisition with a commercial instrument. This is because the detector saturation\textsuperscript{[16,17]} that may occur in the presence of abundant matrix-derived ions can be an obstacle to the highly sensitive detection of analyte ions with a large \textit{m/z} value. As a simple remedy to this problem, matrix-derived ions are routinely eliminated by a deflector.\textsuperscript{[18,19]} This suggests that the performance of an instrument may be somewhat compromised when the abundances of both the matrix and analyte ions, which are required for the peptide quantification in our method,\textsuperscript{[12]} are measured by one and the same detector. An easy solution to this potential problem is to use two detectors, one for the detection of the analyte ions and the other for that of the matrix ions.

In this work, we used the usual detector in a MALDI-tandem TOF instrument that detects ions reflected by reflectron. We also installed an additional detector to detect the matrix ions deflected by the deflector. The design and operation of the instrument including the method\textsuperscript{[20]} to control the effective temperature and its performance in the peptide quantification will be presented. Also, it will be demonstrated that the instrument retains its capability as a tandem TOF and hence can assist with the identification of an analyte(s) in a complex mixture.

A MALDI-tandem TOF constructed previously\textsuperscript{[21]} was converted into a dual track instrument in this work. A schematic drawing of the instrument is shown in Fig. 1. The original instrument consisted of a MALDI source with delayed extraction, a linear TOF analyzer, an ion gate, a reflectron, and a multichannel plate detector (MCP, \#31849, Photonis USA, Sturbridge, MA, USA). The distances between the source exit and the ion gate, that between the ion gate and the reflectron entrance, and that between the reflectron exit and the detector (to be called MCP1) were 880, 350, and 20 mm, respectively.

In the modified instrument, a simple bipolar deflector was installed on the ion optical axis 430 mm from the source exit. Taking \(x\) as the ion optical axis and \(xy\) as the plane on which MCP1 was installed, ion deflection by the deflector occurs along the \(y\) axis, away from the ion gate. The second MCP (MCP2) was installed on the \(xy\) plane also, at 100 mm after the ion gate and at \(y\) of 50 mm from the ion optical axis (see Fig. 1). It should be noted that the position of MCP2 along the \(x\) axis corresponds to the first time focal point in the original instrument. The voltages on the deflector were adjusted so that the deflected ion beams arrive nearly at the center of MCP2. These were +1.2 and −1.2 kV when the average translational kinetic energy of the ions coming out of the source was 21.5 kV.

For MALDI, the 337 nm output from a nitrogen laser (MNL 100, Lasertechnik Berlin, Berlin, Germany) focused by a lens (\(f = 100\) mm) was used. In a typical measurement, −2.08 and −1.32 kV were applied to MCP1 and MCP2, respectively.

The instrument can be run in two different modes: the ordinary mass spectrometric and tandem mass spectrometric modes. Schematic drawings of the two pulsing schemes are shown in Fig. 2. In the ordinary mass spectrometric mode (Fig. 2(a)), the ion gate is grounded throughout a run. A run starts with the deflector turned on. Then, it is turned off at 5.7 \(\mu\)s after MALDI laser irradiation, changing the ion track from the linear to the reflectron one. At 1 ms (or shorter) before irradiation by the next laser pulse, the deflector is turned back on. Then, the instrument is ready for the next run.

A tandem mass spectrometric run (Fig. 2(b)) also starts with the deflector turned on, and it is turned off at 5.7 \(\mu\)s after laser irradiation, just as in the ordinary mass spectrometric run. The voltage on the ion gate, which is kept on at the beginning of a run, is turned off when a peptide ion of interest approaches and is then turned back on to deflect away ions with \textit{m/z} values larger than the ion of interest. It should be noted that the ions appearing in a spectrum acquired in the reflectron mode consist of the ion of interest and its post-source decay (PSD)\textsuperscript{[22]} product ions.

There is another way of running the instrument in the ordinary mass spectrometric mode, i.e. using the reflectron track for the analysis of matrix-derived ions and the linear track for that of analyte-derived ions. For example, the linear

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{A schematic drawing of the dual track MALDI-TOF instrument.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Schematic drawings of the pulsing schemes for (a) ordinary and (b) tandem mass spectrometric modes. See text for details.}
\end{figure}
track can be used for the detection of high m/z ions, such as those of proteins. Here, a run starts with the deflector turned off. 14.7 μs after laser irradiation, the deflector is turned on and sends ions to the linear track.

EXPERIMENTAL

Data acquisition and treatment

It is known that the fluorescence of a MALDI laser is a factor that affects the ion yield\(^{[21]}\) – we speculate that it affects the ion yield indirectly, i.e., by affecting the surface temperature of an irradiated sample.\(^{[9]}\) With the focusing optics fixed, the laser fluorescence is nearly proportional to its pulse energy. Hence, we will quote the pulse energy whenever needed rather than the fluorescence, specifically as a multiple of the threshold value. Previously, we defined the threshold as the pulse energy at which an ion(s) was observed in 50% of the shots.\(^{[11,24]}\) It was 0.30 μJ per pulse for MALDI using α-cyano-4-hydroxycinnamic acid (CHCA) as a matrix.

Spectral data from every 10 laser shots were averaged. The area of each peak calibrated for the detector gain\(^{[25]}\) was taken as the abundance of the corresponding ion. \(T_{\text{early}}\) was estimated from the fragmentation pattern of the matrix ion in each spectrum (to be explained later).\(^{[12]}\) The method used to acquire a temperature-controlled MALDI spectrum will also be explained later.\(^{[20]}\)

Samples

\(Y_5K\) and angiotensin II (DRVYIHPF) were purchased from Peptron (Daejeon, Korea). A protein mixture (protein standard II containing trypsinogen, protein A, and bovine serum albumin (BSA)) was purchased from Bruker Daltonik GmbH (Bremen, Germany). Cytochrome c and CHCA were purchased from Sigma (St. Louis, MO, USA). An aqueous solution of a peptide was mixed with a 1:1 water/acetonitrile solution of CHCA. 1.0 μL of a solution containing 0.03–30 pmol of the peptide and 25 nmol of CHCA were loaded and vacuum-dried.

RESULTS AND DISCUSSION

Acquisition of temperature-controlled MALDI spectra

Previously, we observed that the MALDI spectrum of a sample was fixed when \(T_{\text{early}}\) was fixed.\(^{[9]}\) We speculated that this correlation arose because the surface temperature after laser irradiation determined both the ion formation process in MALDI and the effective temperature in the early plume (\(T_{\text{early}}\)) where in-source decay (ISD)\(^{[26]}\) occurred.\(^{[12]}\) We also found that the total ion abundance (TIC) in a spectrum correlated with \(T_{\text{early}}\).\(^{[9,20]}\) Based on this, we developed the following method to acquire temperature-controlled MALDI spectra.\(^{[50]}\) We record a spectrum and measure TIC in the spectrum. When TIC is larger (or smaller) than a preset value, we reduce (or raise) the laser pulse energy by rotating the variable neutral density filter (model CNDQ-4-100.0 M, CVI Melles Griot, Albuquerque, NM, USA) that is used to adjust the pulse energy. We demonstrated that the temperature-controlled spectra thus acquired were quite reproducible throughout a measurement.\(^{[20]}\) We also reported calibration curves for some peptides obtained under the temperature control, which were linear over wide dynamic ranges.\(^{[20]}\)

It is somewhat inconvenient to implement the above method, i.e., temperature control via TIC control, in dual track TOF spectrometry mainly because the ion signals in each spectrum are detected by two MCPs with different and variable gains. In addition, some ions are difficult to detect due to pulser noises. Before the development of the TIC-control method, we utilized the abundance ratios for some matrix-derived ions, e.g., the \([M+H−H_2O]^+/[M+H]^+\) ratio, as measures of \(T_{\text{early}}\).\(^{[12]}\) This method would be easier to implement for dual track TOF because it utilizes the outputs of only one MCP. In this work, we took the \([\text{CHCA}+H−\text{CO}_2]^+/[\text{CHCA}+H−\text{H}_2\text{O}]^+\) abundance ratio as the measure of \(T_{\text{early}}\) and used it for the feedback control of \(T_{\text{early}}\) by adjusting the transmission of the optical density filter. This ratio vs. \(T_{\text{early}}\) in CHCA-MALDI is shown in Supplementary Fig. S1 (Supporting Information).

Previously, we also reported that \(T_{\text{early}}\) kept decreasing upon the repetitive irradiation of a spot on a sample.\(^{[8]}\) In the temperature-controlled mode of spectral acquisition, this forced a steady increase in the laser pulse energy as the shot continued.\(^{[20]}\) For example, when the spectral acquisition from a spot started at three times the threshold in CHCA-MALDI, the pulse energy went up to five times the threshold by the time the spot was almost completely depleted by repetitive irradiation, which occurred after approximately 300 shots. To avoid collecting data from a spot where some parts were completely depleted, we stopped data collection when the pulse energy became 3.5 times the threshold. The temperature-controlled CHCA-MALDI spectra of \(Y_5K\) in shot number ranges of 31–40 and 101–110 acquired from a spot are shown in Fig. 3. Each spectrum was obtained by stitching together the spectra acquired by the two detectors. Important matrix- and analyte-derived ions are marked in the spectra. It should be noted that the two spectra are virtually the same, as was found in previous studies.\(^{[9,20]}\)

Conducted with single track instruments. Also shown in the figure are the spectra in the same shot number ranges acquired with the tandem mass spectrometric mode of operation. Here again, the two spectra obtained in different shot number ranges are virtually the same. Activation of the ion gate in this mode of operation eliminated most of the ion signals detected by MCP1. Ions appearing in the tandem mass spectrum thus acquired were mostly \([Y_5K+H]^+\) and its ISD products, e.g., \(a_n\) (\(n = 2–5\)), \(b_n\) (\(n = 2–5\)), and \(y_n\) (\(n = 1–5\)). It should be emphasized that the tandem mass spectrometric operation of a dual track TOF can be useful for the identification of peptides.

Calibration curve

In our previous studies with a single track MALDI-TOF equipped with a reflectron, we reported direct proportionality between \(I(P+H')/I([M+H']^+)\) and \(c(P)\) for all of the peptides investigated.\(^{[12]}\) In this work, we operated the dual track TOF in the tandem mass spectrometric mode with MCP1 and MCP2 detecting analyte- and matrix-derived ions, respectively. \(I(P+H')/I([M+H']^+)\) vs. \(c(P)\) data thus obtained for samples containing 0.03–30 pmol of \(Y_5K\) in 25 nmol of CHCA displayed excellent linearity, as shown in Fig. 4. A similar result for angiotensin II is shown in the same figure. We also
obtained calibration curves with the instrument operating in the ordinary mass spectrometric mode. The results shown in Supplementary Fig. S2 (Supporting Information) are comparable to those in Fig. 4.

Linear TOF for protein ions and reflectron TOF for matrix-derived ions

It is well known that ion signals of high-mass proteins detected in the reflectron mode are significantly weaker than those detected in the linear TOF mode. Unless a very high resolution TOF is used, the reflectron mode is not useful for resolving the isotopic peaks for a protein either. Hence, it is common to detect protein ion signals in the linear mode, often utilizing an MCP installed after the final electrode of the reflectron. In such cases, use of a high gain for MCP is imperative, which necessitates the elimination of matrix-derived ions by de-fection. An alternative is to utilize a dual track TOF and analyze protein ions and matrix-derived ions by the linear (MCP2) and reflectron (MCP1) modes, respectively. A CHCA-MALDI spectrum of a sample containing trypsinogen, protein A, and BSA acquired by the dual track TOF is shown in Fig. 5. Another spectrum acquired from a sample containing cytochrome c is shown in Supplementary Fig. S3 (Supporting Information). The early associated with the spectrum can be estimated from the \([\text{CHCA} + \text{H} – \text{CO}_2]^+ / [\text{CHCA} + \text{H} – \text{H}_2\text{O}]^+\) ion abundance ratio of 0.2 was used for temperature control. Two spectra acquired by MCP1 and MCP2 have been stitched together. ISD and PSD peaks are marked with open (○) and filled (●) circles, respectively.

Figure 3. Temperature-controlled CHCA-MALDI spectra acquired with the ordinary mass spectrometric mode from a spot on a sample of 3 pmol Y5K in 25 nmol CHCA in shot number ranges of (a) 31–40 and (b) 101–110. Those obtained with the tandem mass spectrometric mode in shot number ranges of (c) 31–40 and (d) 101–110 are also shown. A \([\text{CHCA} + \text{H} – \text{CO}_2]^+ / [\text{CHCA} + \text{H} – \text{H}_2\text{O}]^+\) ion abundance ratio of 0.2 was used as the preset value for temperature control. Two spectra acquired by MCP1 and MCP2 have been stitched together.

Figure 4. Calibration curves for (a) Y5K and (b) angiotensin II plotted as \(I([P + \text{H}]^+)/I([M + \text{H}]^+)\) vs. \(c(P)\) data obtained with the tandem mass spectrometric mode for samples containing 0.03–30 pmol of analyte in 25 nmol of CHCA. A \([\text{CHCA} + \text{H} – \text{CO}_2]^+ / [\text{CHCA} + \text{H} – \text{H}_2\text{O}]^+\) ion abundance ratio of 0.2 was used for temperature control. Error bars indicate one standard deviation.
the present work. However, we have not been successful yet in collecting data that display a systematic trend in protein MALDI.

Thus far, we have shown that dual track TOF MS can be useful for detecting matrix- and peptide-derived ions without the complication of detector saturation. In principle, the same can be achieved with the usual single track TOF MS by applying a low voltage to MCP1 when matrix-derived ions arrive and then switching it to a high voltage when peptide-derived ions arrive. We found that the MCP gain could be made to fully recover within 1 µs after voltage switching (unpublished data) and hence that the above method could be used to avoid detector saturation. However, the method turned out to be more inconvenient to implement. For example, the ion gate had to be turned on and off twice during one tandem mass spectrometric run.

CONCLUSIONS

Detector saturation by abundant matrix-derived ion signals is one of the inconveniences encountered when acquiring reproducible spectra in MALDI-TOF. In this work, we developed a fairly straightforward method of avoiding this difficulty, i.e. utilization of two detectors, one for matrix-derived ions and the other for peptide-derived ions. With the matrix and peptide ion abundances in the spectra thus acquired, excellent linear calibration curves for peptides were obtained. These can then be used for their quantification.

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REFERENCES


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