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Desorption/Ionization on Silicon Time-of-Flight/ Time-of-Flight Mass Spectrometry

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Desorption/ionization on silicon (DIOS) tandem time-of-flight (TOF/TOF) mass spectrometry (MS) provides high accuracy and significant fragmentation information, particularly in the characterization of biomolecules. DIOS TOF/TOF offers a high-throughput surface-based ionization platform as well as complete fragmentation through high collision energies. The absence of matrix interference in DIOS allows for the MS and MS/MS analysis of small molecules well below *m*/*z* 300. In addition, sample preparation is minimal, and the DIOS chips can be stored and reanalyzed for fragmentation information or accurate mass measurements. The combined benefits of robustness, minimal sample preparation, good sensitivity, high throughput, and sequencing capability make DIOS TOF/TOF a powerful tool for small molecule characterization and protein identification.

Matrix-assisted laser desorption/ionization (MALDI) tandem time-of-flight (TOF/TOF) mass spectrometry (MS) has been recently recognized for its ability to rapidly provide high accuracy and high energy fragmentation information, particularly in the characterization of proteins.[1−5] Current tandem mass spectrometers include quadrupole ion traps, ion cyclotron resonance, triple quadrupoles, or hybrid quadrupole time-of-flight analyzers, all of which yield low energy fragmentation through collision-induced dissociation and are generally coupled with electrospray ionization (ESI). The MALDI TOF/TOF offers both a high-throughput surface-based ionization platform and higher collision energies, which yield more complete structural information from singly charged ions.[1,5] In addition, this instrumentation is equipped with a high-speed sample stage and a Nd:YAG laser operating at 200 Hz, thus allowing for very fast scan rates and rapid sample analysis. In a recent application, Bienvenut et al. utilized MALDI TOF/TOF for protein identification via analysis of a tryptic mass map where the increased structural information gained from the use of this technique lead to the identification of a rare posttranslational modification on tryptophan.[3]

While MALDI offers an attractive alternative to ESI for high-throughput tandem MS analysis, the working mass range is limited by matrix interference and is generally bound at the low mass range by $m/z \sim 700$. Desorption/ionization on silicon (DIOS) is a matrix-free technique, where analyte molecules are trapped within a porous silicon surface from which they are laser desorbed and ionized.[6−8] The absence of matrix interference allows for the analysis of small molecules below m/z 300. In addition, the sample preparation is minimal and samples can be stored on DIOS chips to be reanalyzed. The combined benefits of robustness, minimal sample preparation, good sensitivity, high throughput, and sequencing capability make DIOS TOF/TOF a powerful tool.[6−8] Specifically, the lower mass range of DIOS offers a clear advantage over MALDI for the tandem MS analysis of small molecules. The data presented here show the first application of DIOS TOF/TOF to both small molecule characterization and protein identification.

EXPERIMENTAL METHODS

DIOS TOF/TOF experiments were conducted on an Applied Biosystems 4700 proteomics analyzer equipped with an ND-YAG laser (355 nm) operating at a repetition rate of 200 Hz. DIOS chips were directly attached to the MALDI target plate with conductive tape. Figure 1 shows a schematic of the DIOS TOF/TOF instrument illustrating its ability to generate fragmentation spectra. The ESI tandem mass spectrometry experiments were performed on a PE Sciex API III triple quadrupole mass spectrometer and an Agilent quadrupole ion trap mass spectrometer.

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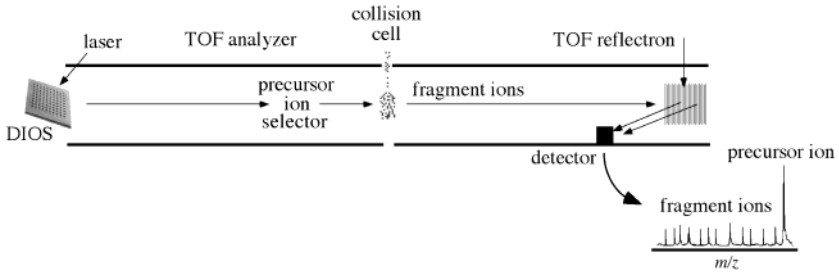

Figure 1. Schematic diagram of the DIOS TOF/TOF system.

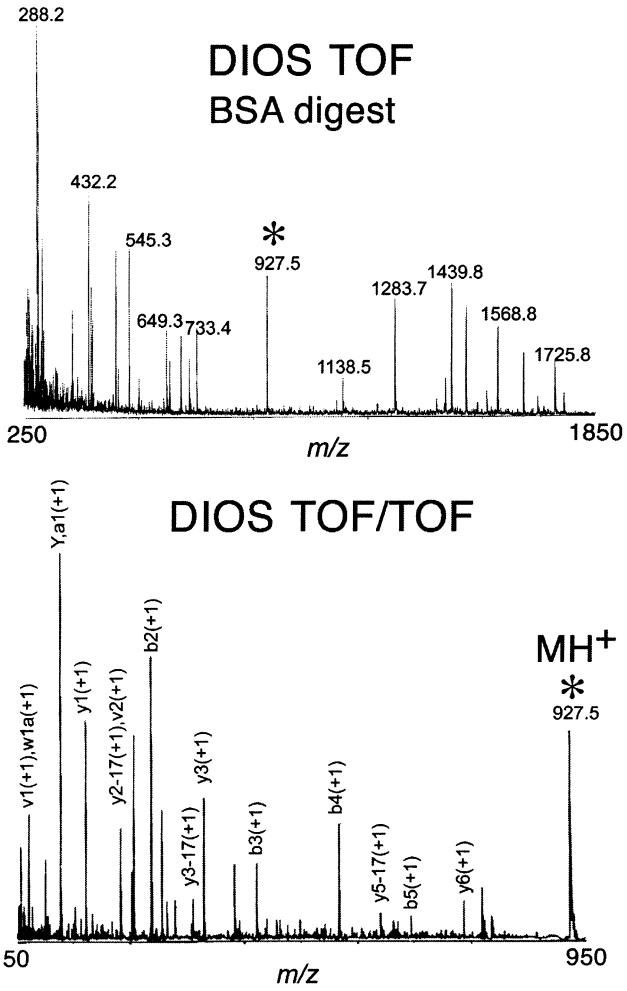

Figure 2. DIOS TOF/TOF spectrum of BSA digest and the TOF/TOF fragmentation mass spectrum of the singly charged ion, $MH^+ = 927.5$.

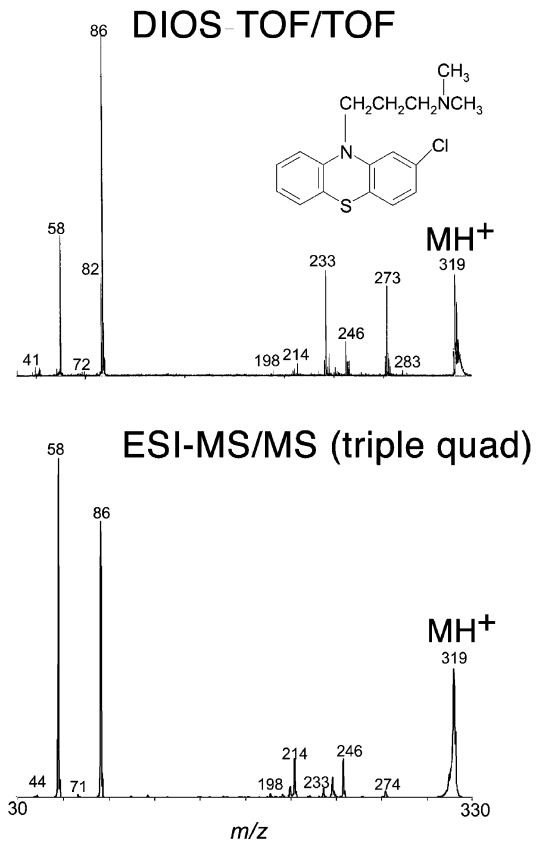

Figure 3. Tandem mass spectral data of cimetidine (MW 252.1) obtained by DIOS TOF/TOF and ESI triple quadrupole.

The details of DIOS chip preparation have been previously published.[6] In brief, DIOS chips were prepared by electrochemical etching of a low-resistivity (0.005−0.02 Ω·cm), n-type Si(100) wafer in a 25% v/v HF/ethanol (Acros and Sigma, respectively) solution under white light illumination for 2 min at a current density of 5 mA/cm^2. Photopatterning in the previous step allows for the analysis of multiple samples on each chip. Following the first etching, the surface was oxidized with ozone and re-etched with 5% v/v HF/H_2O solution.

Aqueous stock solutions of chloropromazine and cimetidine (Sigma) were prepared at 50 μM. BSA (Sigma) proteolytic digests were prepared with trypsin (Promega, 1:50 enzyme-to-protein ratio by mass). The BSA tryptic digest was incubated overnight at 37 °C in 5 mM ammonium citrate buffer (Sigma, pH 7.5). The enzymatic reaction reached completion within 18 h, yielding a final BSA concentration of 0.2 μM. Samples (0.5 μL) were pipetted directly onto the DIOS chip.

RESULTS AND DISCUSSION

DIOS TOF/TOF experiments were performed on a model protein system (BSA tryptic digest, 100 fmol) to illustrate the effectiveness of this technique for protein characterization. The resulting MS and MS/MS (YLYEIAR, $MH^+ = 927.5$) spectra are shown (Figure 2). The data in the low mass region provided complete detection of the y and b ion series and the high energy fragment ion, Y. For comparison, a parallel LC−MS/MS experiment was also performed using an ESI ion trap system (not shown). The primary difference between the two spectra is the absence of high-energy fragment ions and the limited lower mass range in the LC−MS/MS results. The rich fragmentation pattern and high accuracy observed in the DIOS TOF/TOF spectrum

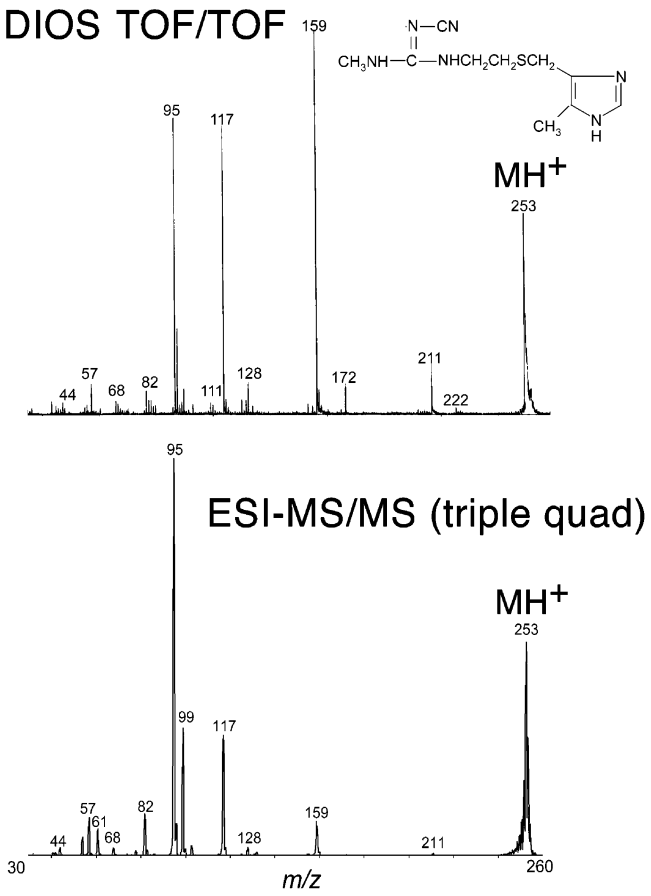

Figure 4. Tandem mass spectral data of chlorpromazine (MW 318.1) obtained by DIOS TOF/TOF and ESI triple quadrupole.

provided a more complete determination of peptide sequence and protein identification.

To illustrate the potential of DIOS TOF/TOF for small molecule analysis, the fragmentation of two model drug molecules were investigated: cimetidine ($C_{10}H_{16}N_6S$, MW 252.1) and chlorpromazine ($C_{17}H_{19}ClN_2S$, MW 318.1). ESI tandem mass spectrometry experiments using a triple quadrupole system were also performed for comparison. The resulting MS/MS spectra from both techniques for each small molecule are shown in Figures 3 and 4. The DIOS TOF/TOF and ESI triple quadrupole fragmentation spectra for each molecule (precursor ions m/z 319.2 for chlorpromazine and m/z 253.1 for cimetidine) are similar. However, it is evident that the DIOS TOF/TOF data contains additional fragmentation information demonstrating the advantage of the higher energy fragmentation.

Aside from the ability of DIOS TOF/TOF to yield informative mass spectral data, the fast sample stage, high repetition rate laser, and TOF/TOF platform also offer the potential for rapid MS and MS/MS analysis that would considerably increase the throughput of DIOS tandem MS analysis. This high-throughput capability is especially significant for small molecule analysis due to the current lack of rapid tandem MS techniques sensitive in the mass region below 300 m/z. With the recent developments in proteomics and increasing interest in metabonomics, high-throughput MS analysis with high accuracy is becoming more important. At present, analyses of such complex biological samples are performed via a combination of multidimensional separations coupled with electrospray ionization MS/MS (ESI-MS/MS) or MALDI-MS.[9,10] The use of DIOS TOF/TOF coupled with 2D-PAGE or on-line/off-line LC separation has the potential of meeting the analysis demands of protein characterization as well as other biomolecule mixtures due to advantages such as ease of sample preparation, improved lower mass range, increased throughput, and enhanced sensitivity.

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