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Application of Molecular Beam Deflection Time-of-Flight Mass Spectrometry to Peptide Analysis

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The application of molecular beam deflection time-of-flight mass spectrometry (MBD-TOFMS) to peptide identification is described. The technique permits a simultaneous measurement of molecular mass and electric dipole susceptibility. The mass and susceptibility are not strongly correlated, and the results can be presented as a two-dimensional map. The susceptibility provides a useful way to disperse isobaric and isomeric peptides, and at least for small peptides, the susceptibility is significantly different for different amino acid sequences. Results for peptides in the mass range 1000−2300 Da show that the mass and susceptibility lead to a higher identification score than mass spectra alone.

Over the past decade, mass spectrometry (MS) methods have achieved high resolution, accuracy, and sensitivity.[1] Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) sources have permitted the analysis of a wide range of biomolecular species,[2,3] and mass spectrometry is now the method of choice for characterizing proteomes.[4] High-resolution mass spectrometry, with a mass accuracy at the ppm level, usually permits the elemental composition to be determined for peptides up to ∼800 Da from the mass alone.[5] Residues with indistinguishable molecular weights, isomeric residues, and stereoisomers are clearly limitations to determining the amino acid composition from the accurate mass, and this is usually restricted to peptides under ∼600 Da; by 1000 Da there are ∼50 000 possible amino acid compositions per mass unit. However, an example of peptide characterization using ultrahigh mass resolution has been reported.[6]

The incorporation of multiple stages of mass spectrometry (MS/MS or MS^n) has provided another approach for biomolecular analysis, since it provides direct information about sequences, especially when coupled with database search algorithms.[7] Protein identification can thus be achieved by analyzing, with multiple stages of MS, either intact proteins ions or peptides derived from proteolytic digestion.[8] However, due to the small number of observed fragments for certain proteins or the large number of different sequences observed at similar masses, mass spectrometry typically fails to identify more than 30% of proteins in routine operations.

Many proteomics applications require the analysis of complex peptide mixtures. In these cases, an alternative strategy is to couple mass spectrometry to another analytical tool, which adds another dimension to the mass measurement to resolve peptides with similar masses. For example, Clemmer and co-workers proposed new experimental interfaces for biomolecule analysis involving ion mobility and mass spectrometry. The mobility of an ion is sensitive to its size and shape, and this allows the separation of peptide ions with the same nominal masses but different shapes.[9] A two-dimensional map of mass and mobility for peptide/protein mixtures leads to a higher identification score than conventional mass spectrometry.[10−12] Ideally, it should be possible to resolve and identify all the sequences with similar masses using the second analytical tool, though this has not yet been achieved.

Optical and electric properties are fundamental characteristics of molecules that are widely used in solution protein studies and could be coupled to mass spectrometry to enhance peptide

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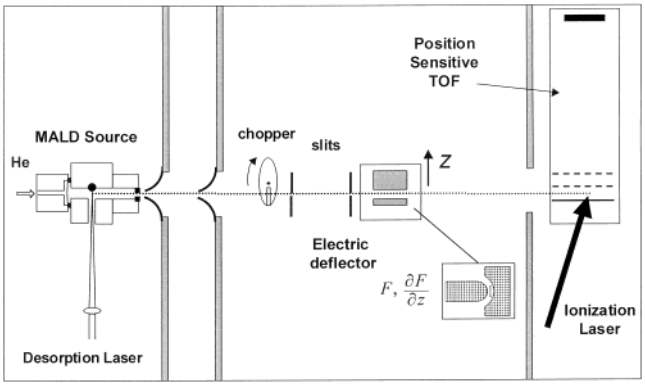

Figure 1. Schematic diagram of the molecular beam deflection time-of flight mass spectrometry experiment combined with a matrix-assisted laser desorption source.

identification. To date, optical spectroscopy on gas-phase biomolecules has been restricted to small systems. In this paper, we examine the possibility of using the electric dipole susceptibility for peptide analysis. The electric susceptibility of a polypeptide is very sensitive to its conformation; for example, a helical conformation has a macrodipole that leads to a very large susceptibility, while the susceptibility of the random coil conformation is much lower.[13]

Recently, we coupled a matrix-assisted laser desorption (MALD) source to a molecular beam deflection time-of-flight mass spectrometer (MBD-TOFMS) experiment and used it to measure the average electric dipole moments of a few small isolated neutral peptides.[14] In this paper, we describe the MBD-TOFMS experiment and its application to the analysis of peptides, and we show that the electric susceptibility can be used to complement mass spectrometry in peptide characterization. The performance of the MBD-TOFMS approach is demonstrated by the analysis of small isobaric neutral peptides generated in the MALD source, as well as for larger peptides in the 1000−2300-Da mass range.

EXPERIMENTAL SECTION

Overview. A schematic diagram of the experimental apparatus is shown in Figure 1. The instrument is composed of three parts: (1) a MALD source; (2) an electric deflector; and (3) a position-sensitive time-of-flight mass spectrometer. Briefly, a pulsed neutral molecular beam travels through the deflector where there is a strong inhomogeneous electric field. One meter after the deflector, the molecular beam is photoionized in the extraction region of a position-sensitive time-of-flight mass spectrometer (PS-TOFMS), which is orthogonal to the molecular beam axis. The arrival time at the detector (i.e., the mass) and the beam profile (i.e., the effect of the electric field) are simultaneously recorded with the PS-TOFMS. All measurements were made by comparing, with the same source conditions, the beam profiles of a peptide without and electric field ($F = 0$ V/m) and with an electric field ($F \neq 0$ V/m) inside the deflector. As seen below, the effect of the electric field leads to global deflection of the beam, which is directly proportional to the electric susceptibility of the peptide in the molecular beam.

Sample Preparation and Beam Source. The peptides were purchased from commercial sources (Sigma, Bachem AG., or Advanced ChemTech) except for WV_n (n =10, 15, 20) and Ac-WV_{20}-NH_2 peptides (W = tryptophan; V = valine), which were synthesized using FastMoc chemistry on an Applied Biosystems model 433A peptide synthesizer, and used without further purification. The peptides are mixed with a matrix in a 1:10 to 1:3 mass ratio and pressed under 10^4 bar in a cylindrical mold to form a solid rod (∼10 mm long, 6-mm outer diameter, 2-mm inner diameter). Typically 1−10 mg of peptide is used to form a rod (in the future it should be possible to develop methods that employ much less sample). A variety of different matrixes were used during this study. Nicotinic acid and especially cellulose gave the best signals. The rod is placed inside the source and is rotated and translated in a screw motion. The molecules are desorbed from the matrix with the third harmonic of an Nd^{3+}:YAG laser (355 nm). A pulsed helium flow generated with a piezoelectric valve is used as the carrier gas. The pulsed valve is synchronized with the desorption laser pulse. A molecular beam of the target peptide leaves the source through a 50-mm-long nozzle. The nozzle diameter is 2 mm, and the source pressure is a few Torr. The beam is then tightly collimated by two skimmers and two slits.

Electric Deflector. The poles of the electric deflector are two 15-cm-long cylinders that are 1.7 mm apart. This geometry provides an inhomogeneous electric field equivalent to a "two-wire" arrangement. The electric field F and the field gradient $\partial F/\partial z$ are perpendicular to the axis of the beam and are nearly constant over its width.[15] For a voltage of 27 kV, the values of the electric field F and of the gradient of the electric field $\partial F/\partial z$ are, respectively, 1.62×10^7 and 2.88×10^9 V/m^2 in the center of the deflector. The voltage V inside the deflector can be adjusted from 0 to 30 kV. In the deflector, a molecule with an electric dipole moment $\vec{\mu}$ is submitted to an instantaneous force along the z axis of $f = \vec{\mu}(\partial\vec{F}/\partial z)$. Assuming a linear response,[16,17] $\langle \mu_z \rangle = \chi(0)F$, and the deflection d of a molecule of mass m and velocity v is given by

$$d = \frac{K}{mv^2}\chi(0)F\frac{\partial F}{\partial z} = \frac{K'}{mv^2}\chi(0)V^2 \qquad (1)$$

where K and K' are geometrical factors that depend on the dimensions of the apparatus. The beam velocity is selected and measured with a mechanical chopper located in front of the first slit.

Position-Sensitive Time of Flight. One meter after the deflector, the molecules are photoionized with the fourth harmonic of a Nd^{3+}:YAG (266 nm) in the extraction region of a position-sensitive time-of-flight mass spectrometer. The pulse width of the laser is 6 ns and its fluence is ∼3 mJ/cm^2. Particular attention is paid to having a homogeneous laser spot (8 mm × 3 mm) near the center of the extraction region. The electric fields in the extraction and acceleration region of the TOFMS are adjusted so that the arrival time of a molecule at the detector (which consists of dual microchannel plates) is sensitive to the initial position of

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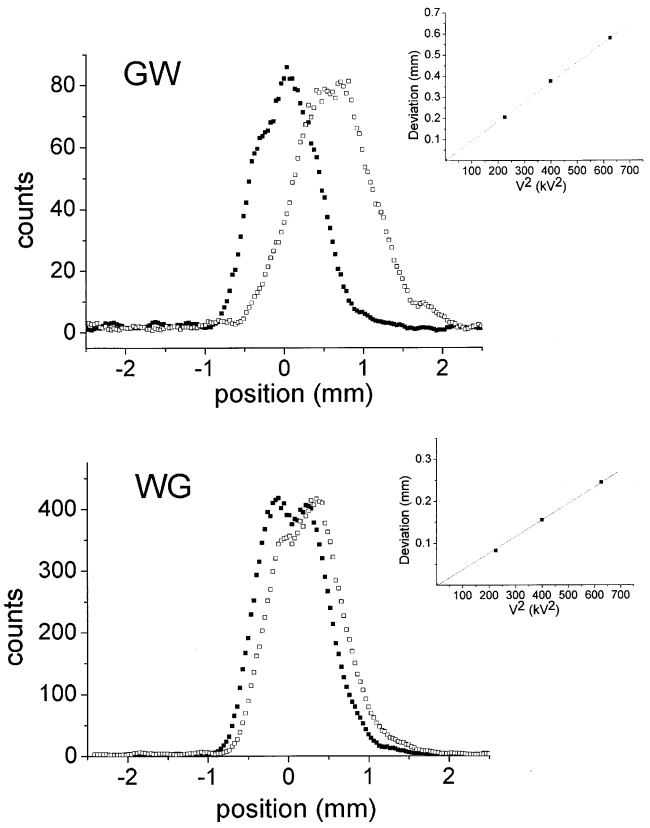

Figure 2. Beam profiles of GW and WG peptides measured without (■) and with (□) a voltage (25 kV) on the deflector. The insets show plots of the deviation of the beam as a function of the square of the voltage on the deflector. The solid lines show linear fits to the data.

the molecule in the extraction region (position-sensitive time of flight).[18] The profile of the beam in the ionization region is determined from the arrival time distribution at the detector. The beam profile is measured as a function of the electric field in the deflector.

RESULTS AND DISCUSSION

Isomeric and Isobaric Peptides. (a) Dipeptides. Figure 2 shows beam profiles measured for isomeric WG and GW peptides (W = tryptophan; G = glycine) with no electric field ($F = 0$ V/m) in the deflector and with $F = 1.5 \times 10^7$ V/m (25 kV). The profiles are nearly symmetric and can be fit with a Gaussian. The peptide beam profiles measured with the electric field are shifted toward the high-field region in the deflector. The deflections measured at different deflector voltages are plotted against the square of the applied voltage in the insets of Figure 2. A linear behavior is obtained, as expected from eq 1. The dc electric susceptibilities $\chi(0)$ are directly obtained from the deflections using eq 1 and are listed in Table 1. The electric susceptibility strongly depends on the sequence of the dipeptide. GW has an electric susceptibility of 547 ± 55 Å^3, which is more than twice the value measured for WG (214 ± 27 Å^3). The same experiment was performed for WA and AW peptides (A = alanine), and the results are listed in Table 1. The susceptibility is again strongly dependent on the sequence. The susceptibilities of WA and WG are similar (within the experimental uncertainties), and this is also true for AW and GW peptides. Tryptophan at the C-terminus leads to a much larger susceptibility than with alanine or glycine at the C-terminus.

Table 1. Electric Susceptibility, Formula, and Mass of Neutral Tryptophan-Based Dipeptides

peptide name	formula	mass (Da)	electric susceptibility (Å^3)
GW	$C_{13}H_{15}N_3O_3$	261.277	547 ± 55
WG	$C_{13}H_{15}N_3O_3$	261.277	214 ± 27
AW	$C_{14}H_{17}N_3O_3$	275.303	537 ± 107
WA	$C_{14}H_{17}N_3O_3$	275.303	245 ± 80
WK	$C_{17}H_{24}N_4O_3$	332.398	441 ± 45
WQ	$C_{16}H_{20}N_4O_4$	332.355	299 ± 35

We performed similar experiments for isobaric WK and WQ peptides (K = lysine; Q = glutamine). The mass difference between these two peptides (0.043 Da) requires a resolving power close to the limit of TOFMS to separate them. However, as seen in Table 1, their electric susceptibility is drastically different; WK has an electric susceptibility ~1.5 greater than that of WQ. The lysine residue has a more flexible side chain than the glutamine residue, which presumably leads to different equilibrium structures with significantly different susceptibilities. In conclusion, WA, WG, WK, and WQ have four distinct electric susceptibility values. The electric susceptibility of dipeptides strongly depends on both the nature and the position of the amino acid residues.

(b) Tripeptides. We now consider the susceptibility of the six isomeric peptides composed of glycine, tryptophan, and tyrosine (GWY, GYW, WYG, WGY, YWG, YGW). Figure 3 shows typical mass spectra recorded for these six neutral tripeptides using photoionization with 266-nm light. The photoionization efficiency is enhanced by the fact that the photon energy is close to the resonant bands of both the indole moiety in tryptophan and the phenyl ring in tyrosine. The same source and ionization conditions were used for all tripeptides. Under these conditions, the parent mass is the dominant peak for all peptides, though varying amounts of fragmentation are observed. Peaks at P − 16 Da and P − 45 Da (P = parent) are observed for all tripeptides and are assigned to the loss of NH_2 and COOH, respectively. Also, fragments are always observed at masses 117, 130, and 131 Da and may be assigned to indole derivatives as already observed in WG_n photoionization mass spectra.[14] Most of the fragments contain the indole residue, which results because fragmentation occurs in the source and the fragments with the indole residue have the highest photoionization cross section. Fragmentation in the source was confirmed by deflection measurements on the fragments (fragments from the source usually have deflections different from that of the parent while fragments generated in the ionization region have the same deflection as the parent). The mass spectra recorded for the tripeptides are different from those observed by MALDI-TOFMS, due to different source conditions and ionization process.

The right-hand side of Figure 3 shows the beam profiles measured for the parents with an electric field $F = 0$ V/m and with $F = 1.5 \times 10^7$ V/m (25 kV) in the deflector. The deflection

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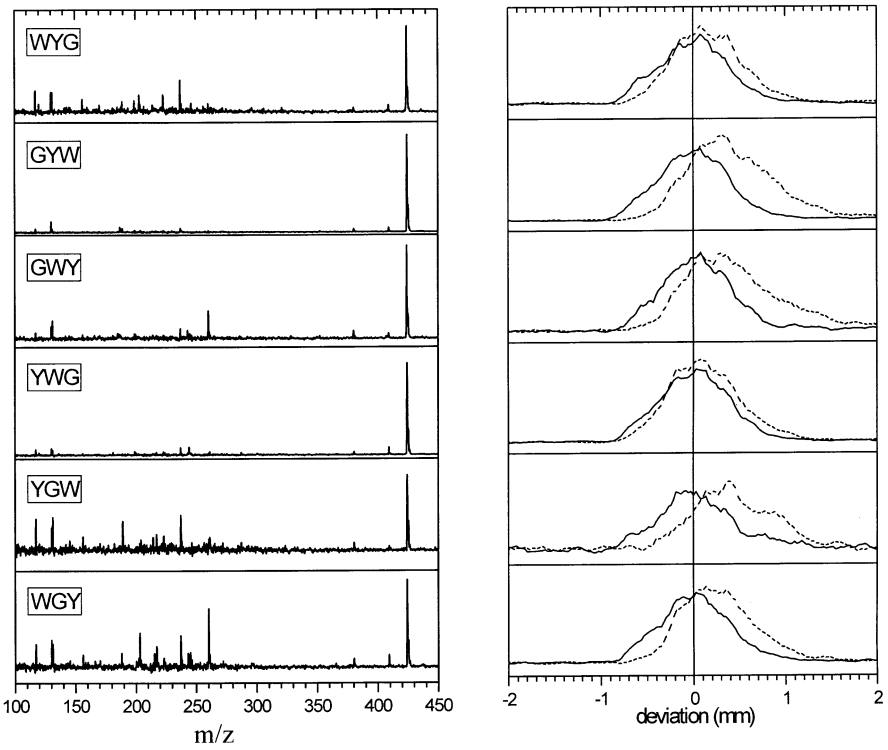

Figure 3. Left-hand side: mass spectra for the six neutral isomeric peptides composed of glycine, tryptophan, and tyrosine (GWY, GYW, WYG, WGY, YWG, YGW) ionized with a photon energy of 4.66 eV (266 nm). Right-hand side: beam profiles of corresponding tripeptides measured without (solid line) and with (dashed line) a voltage (25 kV) on the deflector.

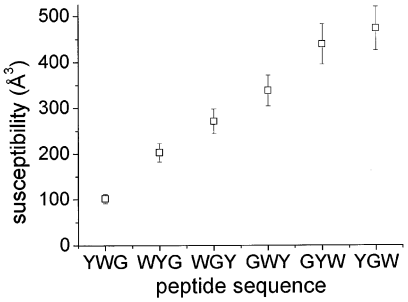

Figure 4. Electric susceptibility of the six neutral isomeric tripeptides as a function of the peptide sequence.

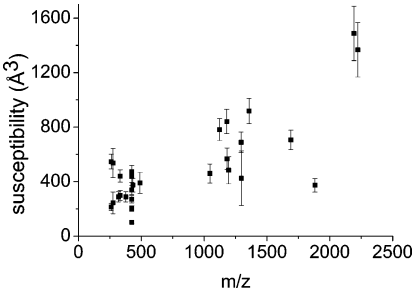

Figure 5. Electric susceptibility of peptides in the mass range 1000−2300 Da plotted as a two-dimensional molecular mass/electric susceptibility map. The results of dipeptides and tripeptides presented in this study and the results for WG_n ($n = 1-5$)[14] have been included.

strongly depends on the peptide sequence. The electric susceptibility is plotted as a function of the peptide sequence in Figure 4. The susceptibilities are in the 100−500-Å^3 range and are different for each peptide. The tripeptides with glycine at the C-terminus have the lowest susceptibilities, whereas those with tryptophan at the C-terminus side have the highest. The similarity with the results obtained for the dipeptides suggests the possibility of deriving an empirical scale to relate the susceptibility with the peptide sequence, at least for the small peptides.

Electric Susceptibilities of Longer Peptides. In addition to dipeptides and tripeptides, a selection of longer peptides in the 1000−2300-Da size range have been studied by the molecular beam deflection mass spectrometry technique. With our source and ionization conditions, only singly charged peptides are observed. The results are plotted in Figure 5, as a molecular mass/electric susceptibility map (MS/MBD map). The MS/MBD map in Figure 5 summarizes all the results presented in this study, along with results for WG_n ($n = 1-5$).[14] Susceptibilities for every peptide in this map are listed in Table 2. This map demonstrates that electric susceptibility is a particularly effective dimension to separate polypeptides because there is not a strong correlation between the mass and susceptibility. For example, the luteinizing hormone and WYWYWYGG peptide are ∼2 Da mass apart and have susceptibilities that are ∼250 Å^3 apart. The same difference is observed between angiotensin I (1296 Da) and WYGGWYG-GWY (1294 Da). This shows that the susceptibility is discriminating even for large peptides, which may display a variety of different conformations.

CONCLUSIONS AND OUTLOOK

We have previously demonstrated that MBD-TOFMS has the capacity to discriminate between different conformations for WG_n peptides ($n = 1-5$). In the present work, we used much more complex peptides sequences incorporating 17 of the natural amino acids and with masses distributed in the range from 1000 to 2300 Da. For all peptides, MBD-TOFMS can be used to record a two-dimensional map of mass and deflection. These maps allow us to

Table 2. Mass and Electric Susceptibility of the Longer Neutral Peptides Used in Used in Figure 5

peptide sequence	name	mass (amu)	electric susceptibility (Å^3)
DRVYIHPF	angiotensin II	1046	460 ± 70
WYWYWYG		1123	782 ± 80
WYWYWYGG		1180	842 ± 90
pEHWSYGLRPG-NH_2	luteinizing hormone	1182	568 ± 80
WVVVVVVVVVV		1195	484 ± 100
WYGGWYGGWY		1294	689 ± 75
DRVYIHPFHL	angiotensin I	1296	425 ± 200
TPDWAEAELKAQ		1358	919 ± 92
WVVVVVVVVVVVVVVV		1691	707 ± 71
HCO−VGALAVVVWLWLWLW-$NHCH_2CH_2OH$	gramicidin D	1880	374 ± 50
WVVVVVVVVVVVVVVVVVVVV		2193	1488 ± 200
Ac-WVVVVVVVVVVVVVVVVVVVV-NH_2		2222	1368 ± 200

separate peptides having similar molecular weights but different sequences. A unique feature of this experiment is that the measurements are performed on neutral gas-phase peptides and this may allow the observation of species different from those observed with MALDI-MS or ESI-MS experiments. MBD-TOFMS may become a useful tool for proteomics. The next step of this work will be a systematic study of peptide mixtures and tryptic digests of proteins.

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