

Optimization of Sample Preparation for Peptide Sequencing by MALDI-TOF Photofragment Mass Spectrometry

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This paper describes the optimization of sample preparation for MALDI 193-nm photofragment ion time-of-flight mass spectrometry to sequence small to medium-sized peptides from peptide mixtures. We show that matrix additives, such as fructose and phenylbutyric acid have a dramatic effect on the abundance of fragment ions observed in the post-source decay spectra. A dried-droplet MALDI matrix consisting of 1:1 α -cyano-4-hydroxycinnamic acid/fructose proves to be an excellent matrix for photodissociation because $[M + H]^+$ ions are formed with low internal energies, and the photofragment ion spectrum contains high abundances of sequence-informative ions. The addition of fructose appears to improve overall sample homogeneity and durability, as compared to conventional α -cyano-4-hydroxycinnamic acid dried-droplet preparations. MALDI-TOF photodissociation is then used to selectively sequence the peptides bradykinin (RPPGFSPFR), des-Arg⁹ bradykinin (RPPGFSPF), and substance P-amide (RPKPQQFFGLM-NH₂) from a mixture of five peptides.

Over the past decade, matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOFMS) has become a powerful tool for many biochemical applications.¹ MALDI is an excellent ionization method for large molecules, especially proteins and DNA,² because the ions are formed with low internal energies. For most compounds, very little fragmentation is observed, and the dominant analyte ion is the protonated molecule, $[M + H]^+$. Numerous papers have addressed issues of matrix-analyte preparation methods that result in improved sensitivity by reducing the internal energies of the ions. For example, Wilkins and co-workers^{3,4} used a method originally discovered by Beavis and co-workers,⁵ which involved mixing

sugars with the sample to increase the stability of protonated peptides and proteins and, thus, enhance the resolution for Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. Becker and co-workers⁶ showed that careful selection of the matrix increases the sensitivity of MALDI-TOFMS analysis of DNA, and they attributed the observed effects to internal energies of the MALDI formed ions. Glückmann and Karas examined the matrix dependence of initial ion velocity and correlated that to the amount of fragmentation induced in the analyte.⁷ Matrixes that produce ions with low initial velocities are generally "hot" (e.g., produce significant metastable decay), but those that form ions with high initial velocities are "cold" (e.g., produce minimal metastable decay).

MALDI-TOFMS has also proven to be a valuable technique for the analysis and characterization of peptides. For example, postsource decay (PSD) focusing of dissociation products from peptide $[M + H]^+$ ions has greatly improved amino acid sequencing for small peptides.^{8–10} The latest generation of reflectron TOF instruments are capable of high-mass resolution ($R > 10\,000$ – $20\,000$), facilitating peptide amino acid determination and protein identification by peptide mass mapping and protein database searching.^{11–13}

Peptide sequencing by MALDI-TOFMS typically involves the use of a timed-ion selector (MS¹)¹⁴ and reflectron (MS²)¹⁵ to collect the postsource decay fragment ion spectrum.^{9,16} PSD occurs when an ion attains sufficient internal energy during the desorption or ionization process to undergo unimolecular decay in the field-free region between the ion source and the entrance to the electric field of the reflectron.¹⁷ Because MALDI-generated peptide ions

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generally have relatively low internal energies, the abundance of unimolecular decay fragments is quite low, and it is not uncommon to observe abundant fragment ions formed by the loss of small neutral molecules, for example, loss of NH_3 or H_2O .¹⁸ Peptide sequencing by MALDI-PSD is further complicated for peptide mixtures. Shields et al. showed that the abundance of PSD fragment ions is much lower for peptides ionized from mixtures relative to neat samples.¹⁹ To compensate for this, it is common practice to increase PSD fragmentation by increasing the MALDI laser energy and choosing sample preparation methods that maximize fragment ion abundance.^{20,21}

One common approach to increase the overall yield of fragment ions relative to PSD is collision-induced dissociation (CID).^{21,22} CID-TOF is performed by allowing the ions to undergo collisions with inert atoms or molecules in the field-free region prior to a reflectron.²¹ Collisionally activated ions have a broad distribution of internal energies; consequently, the activated ions dissociate via a number of different reaction channels. High-energy, single-collision (keV) CID tends to enhance the yield of low-mass-fragment ions, although it may decrease or suppress entirely the appearance of higher mass metastable fragments.²³ This makes CID less suited to peptide sequencing, because larger-mass-fragment ions are useful in reconstructing the peptide sequence. Perhaps most troublesome for CID-TOFMS is the fact that collisional activation involves conversion of kinetic energy into internal energy. Consequently, following collisional activation, the ions have a range of velocities, giving rise to a broad distribution of arrival times, thus limiting both m/z resolution and mass measurement accuracy.

An alternative method of ion activation which circumvents the limitations inherent to PSD and CID is photodissociation.^{24,25} In photodissociation, the ion of interest is excited by absorption of one or more photons, resulting in unimolecular decay to a fragment ion and neutral.²⁶ Photodissociation imparts well-defined quantities of energy to the ion. Unlike PSD, photodissociation is independent of the desorption process; thus, photofragment ions should be observed in high relative abundance from peptide mixtures. And unlike CID, the kinetic energy distribution of the $[\text{M} + \text{H}]^+$ ion is not changed by absorption of a photon; thus, resolution and mass accuracy for measuring the photofragment ions is identical to that for PSD spectra of metastable ions. Moreover, the energy deposition is controlled by the wavelength of the photon and the chromophores present in the ion, making photodissociation a much more specific method of excitation.^{27–29}

Our efforts have focused on evaluating the utility of photodissociating peptides using excitation from a 193-nm ArF excimer laser,^{30,31} primarily because 193-nm photons are absorbed directly by the amide bond and contain sufficient energy (6.43 eV, 148 kcal mol⁻¹) to cleave a number of bonds. In a previous study, we demonstrated the utility of 193-nm photodissociation delayed extraction (DE) TOFMS for peptide sequencing³¹ and showed that side-chain cleavage reactions were observed.

This paper describes our efforts to optimize sample preparation for photodissociation in two important areas. First, because photodissociation is a signal averaging technique, it is important that samples be homogeneous in terms of $[\text{M} + \text{H}]^+$ ion production. Hercules and co-workers investigated the effect of the matrix additive fucose on shot-to-shot reproducibility.³² Here, we examine two matrix additives that have been utilized in our laboratory in the past, phenylbutyric acid and fructose. Most overlay sample preparation methods are depleted after a few laser shots, thus necessitating several sample spots to acquire a complete photofragment ion spectrum. Our optimized sample preparation procedure allows multiple photofragment ion spectra to be acquired from a single sample spot.

The second important consideration for photofragment MALDI matrixes is the internal energies of the $[\text{M} + \text{H}]^+$ ions; that is, metastable decay of the $[\text{M} + \text{H}]^+$ ion must not complicate the photofragment ion spectrum. It is important to eliminate as completely as possible the PSD signal from the photofragment ion spectrum for several reasons. First, if any quantitative data is to be gathered, such as branching ratios for photofragment pathways, a PSD signal will result in positive error. In addition, if the internal energy of the $[\text{M} + \text{H}]^+$ ions is high, the most abundant ions in the fragment ion spectrum are the low m/z immonium ions, thus decreasing the structurally significant information contained in the photofragment ion spectrum. Stimson and co-workers³³ demonstrated increased occurrence of side chain cleavage in MALDI-CID-TOF using “cold” matrixes. Data are presented which demonstrate the ability to photodissociate peptides from mixtures using optimized photodissociation samples.

EXPERIMENTAL SECTION

Apparatus. All spectra were acquired on a home-built tandem MALDI-TOF mass spectrometer (Figure 1) designed specifically for photodissociation studies. The instrument is designed as a true tandem TOF spectrometer. That is, the ions are temporally focused at the point of intersection of the photodissociation laser and ion beams, thus MS^1 is a linear TOF with a flight path length of 0.58 m. The point of intersection also serves as the image focal point for the reflectron (MS^2) TOF. The apparatus has been described in detail elsewhere,¹⁸ but will be briefly reviewed here.

The MALDI-TOF mass spectrometer has a total ion flight path of 3.9 m and is equipped with a reflectron and delayed extraction. The reflectron is 0.5 m in length and is positioned 1.5° off axis

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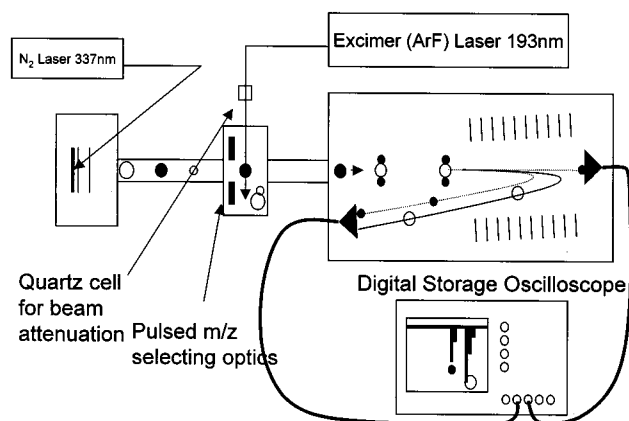


Figure 1. Diagram of the home-built DE MALDI-TOFMS for photodissociation studies. The distance from the source to the ion selection optics and photodissociation region are 0.52 and 0.58 m, respectively. The distance from the ion selection region to the entrance of the reflectron is 0.9 m.

with respect to the incident ion beam. Ions of interest are mass-selected by a parallel plate timed-ion selector positioned 0.52 m from the sample surface in the first field-free region. The reflected ion detector consists of two 40-mm microchannel plates (dual MCP) in a chevron configuration located 0.5 m from the end of the reflectron. Data are acquired on a 1 GHz digital storage oscilloscope (LC574AM, LeCroy Corp, Chestnut Ridge, NY) interfaced to a 100 MHz Pentium PC. In the current experimental configuration, an accelerating voltage of 20 kV with a grid ratio of 0.935 is used, and the extraction grids are pulsed after a delay time of 300 ns.³⁴

Sample desorption/ionization is performed using a nitrogen laser (VSL 337ND, Laser Science, Inc., Franklin, MA) at 337 nm. The output of the laser passes through a mechanical iris and a neutral density filter before irradiating the sample surface at an angle of 20° from normal. During photodissociation studies, the neutral density setting for the desorbing laser is kept at a constant value slightly above the threshold for $[M + H]^+$ ion production.

The output of a precisely timed excimer laser (LPX-120i, Lambda Physik, Acton, MA) intersects the ion of interest in the mass spectrometer at a distance of 0.58 m from the sample surface. To define the dimensions of the excimer beam, it is passed through a mechanical iris 0.4 m from the instrument. The timing between the pulse of the ion extraction optics and the firing of the excimer is stable to ± 2 ns.³⁰ The excimer laser (ArF) is operated in constant high-voltage mode at 25.0 kV, resulting in laser pulse energies of ~ 140 mJ. The laser pulse is 17 ns fwhm. The beam is attenuated to ~ 5 –10 mJ prior to entering a methanol/water variable attenuator, which allows the laser beam energy to be precisely tuned. Spectra in this work were recorded using a methanol/water solution ($\chi_{\text{methanol}} = 0.0025$), which studies in our laboratory have shown to optimize sequence-informative fragmentation. The methanol/water attenuator functions by absorbing some fraction of the incident excimer radiation, thereby decreasing the laser pulse energy. At high laser energy, significant numbers of multiphoton absorption events occur, resulting in formation of structurally uninformative fragment ions, for example,

immonium ions. At lower laser energy, the incidence of multiphoton absorption events is decreased, and single photon photodissociation products, for example, backbone cleavage, predominate. The effect of attenuation of excimer radiation on the photofragment ion spectrum is the topic of another study.³⁵ Laser beam energy measurements were performed using a pyroelectric joulemeter (Vector D200P, Scientech, Inc., Boulder, CO).

Reagents. Bradykinin (RPPGFSPFR; $[M + H]^+$ $m/z = 1060.6$), des-Arg⁹ bradykinin (RPPGFSPF; $[M + H]^+$ $m/z = 904.47$), substance P-amide (RPKPQQFFGLM-NH₂; $[M + H]^+$ $m/z = 1347.73$), angiotensin III (RVYIHPF; $[M + H]^+$ $m/z = 931.51$), and splenin fragment 32–36 (RKEVY; $[M + H]^+$ $m/z = 694.39$) were obtained from Sigma Chemical (St. Louis, MO). α -Cyano-4-hydroxycinnamic acid (HCCA), fructose, and phenylbutyric acid (PBA) were obtained from Aldrich Chemical (Milwaukee, WI). All reagents were used without further purification.

Three stock matrix solutions were prepared: (1) 30 mg/mL HCCA/methanol, (2) 30 mg HCCA + 22 mg fructose/mL methanol, and (3) 30 mg HCCA + 26 mg PBA/mL methanol. Peptide stock solutions were prepared by dissolving 5 mg of peptide in 1 mL of water. Dried-droplet samples were prepared by depositing 1 μ L of sample (43:43:13:1 water/methanol/matrix solution/peptide solution) on the sample stage and allowing them to air-dry. Overlay samples were prepared by depositing 2 μ L of stock matrix solution on the sample stage and allowing it to air-dry. A 1- μ L of sample (63:24:6:7 water/methanol/matrix solution/peptide solution) was then deposited on this "matrix under-layer", allowed to air-dry, and introduced to the mass spectrometer.

Data Analysis. Data analysis was performed using GRAMS software (Galactic Industries Corp., Salem, NH). Each photofragment ion spectrum was acquired at the PSD mirror ratio of interest then truncated and calibrated using eq 1.

$$m_f = m_p \frac{\left(\frac{T_f}{T_p} - C_1\right)}{(1 - C_1)} (\text{MirrorRatio} - C_3(1 - \text{MirrorRatio})^{C_4}) \quad (1)$$

Eq 1 is taken from the Voyager Biospectrometry Workstation User's Guide (PerSeptive Biosystems, Inc., Framingham, MA), and m_f is the mass of the fragment, m_p is the mass of the parent, T_f is the time of flight of the fragment, T_p is the time of flight of the parent, and C_1 , C_3 , and C_4 are constants characteristic of the mass spectrometer.³⁶ It should be stressed that Equation 1 holds for a single-stage, gridded reflectron. Individual spectra are subsequently spliced together to create a complete photofragment ion spectrum.^{8,21}

RESULTS AND DISCUSSION

Optimization of the MALDI Matrix. Previous studies have demonstrated that matrix additives, such as carbohydrates, reduce the shot-to-shot variations of MALDI ion yields as well as decrease the relative amount of internal energy imparted to the ions.^{3–5,32,37,38}

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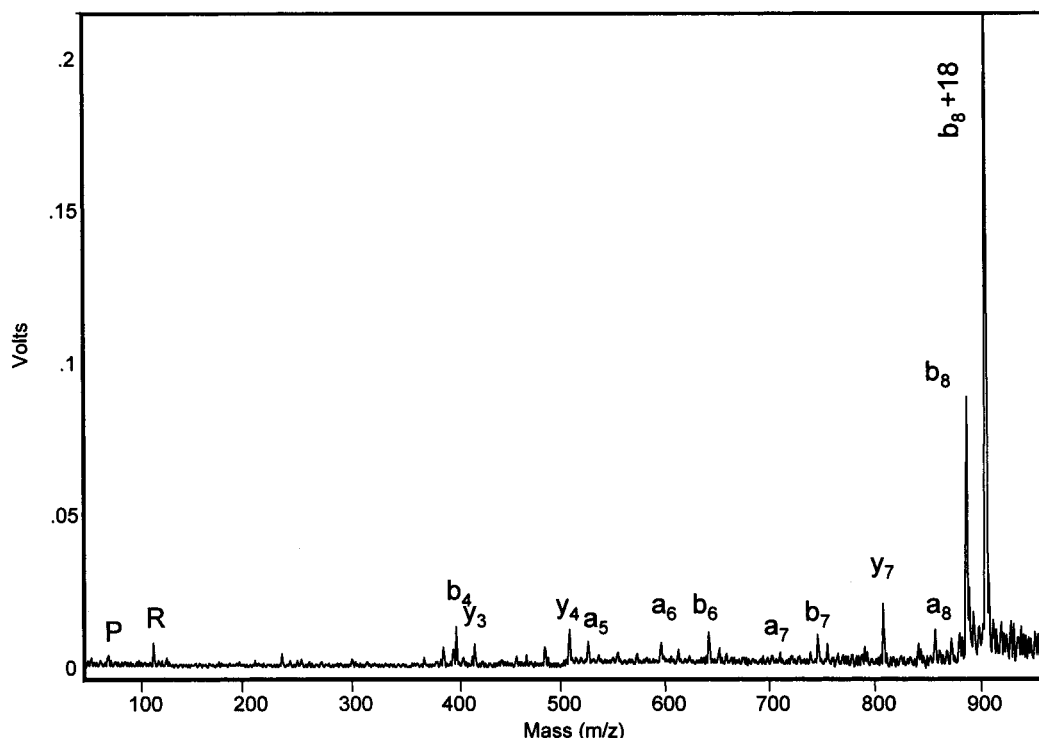


Figure 2. Postsources decay spectrum of bradykinin from an HCCA dried droplet.

Table 1. Shot-to-Shot Precision Data for Two Sample Preparation Methods with Three Different Matrix Compositions^a

sample prep methodology	peak area (arb. units)	rel std dev	peak height (arb. units)	rel std dev
HCCA overlayer	19.35	0.31	13.89	0.41
HCCA/fructose overlayer	5.257	0.254	5.529	0.115
HCCA/PBA overlayer	6.072	0.077	5.488	0.089
HCCA dried droplet	36.77	0.55	35.61	0.47
HCCA/fructose dried droplet	7.371	0.356	0.8020	0.2896
HCCA/PBA dried droplet	15.67	0.43	1.102	0.323

^a Peak height and area data from MALDI-TOF spectra of bradykinin $[M + H]^+$ ions.

In this study, we examine both the dried-droplet and overlayer sample preparation methods using three different matrix and additive combinations, for example, HCCA alone, a mixture of HCCA/fructose, and a mixture of HCCA/phenylbutyric acid.³⁹ Each matrix is evaluated in terms of precision of ion yields and relative internal energy (based on fragment ion abundance measured by PSD) of the $[M + H]^+$ ions. α -Cyano-4-hydroxycinnamic acid was chosen as the matrix for this study, because it gives good signal intensity for a broad range of analyte molecules. Furthermore, it is commonly referred to as a "hot" matrix⁴⁰ (inducing significant metastable fragmentation), thus the cooling effect of matrix additives and ionization of peptide mixtures is clearly illustrated.

It is important that MALDI samples for the photodissociation experiment (and indeed for any quantitative analysis) are homogeneous. If a nonhomogeneous sample spot is used for photodissociation, regions of high and low analyte concentration will be manifested as correspondingly high and low fragment ion currents while sampling those regions. Although this may not be of concern from a sequencing standpoint (where the identity of peaks rather than the intensity is of concern), if any quantitative data is desired, inhomogeneity will be propagated to fragment ion intensities. To determine the overall variability of peptide signal from each matrix and sample preparation procedure, five spectra were recorded from different regions of the sample spot, each an average of 100 laser pulses (rastered over a small localized area). Peak height and area data were obtained for the bradykinin ^{12}C monoisotopic $[M + H]^+$ ion and is presented in Table 1.

The overlayer method gives better overall shot-to-shot reproducibility in terms of ion yields than does the dried droplet method for each matrix composition, suggesting that dried droplet samples are less homogeneous. However, addition of fructose or phenylbutyric acid (PBA) to either matrix preparation further improves the shot-to-shot precision of ion yields. The most significant disadvantage to the overlayer sample preparation is that the laser spot must be continually moved, because the sample is ablated within a few (<10) laser pulses. This is less of a problem with the conventional dried-droplet sample preparation, because up to several hundred spectra may be recorded without moving the laser spot. The disadvantage of dried-droplet samples is that the analyte appears to be highly localized within the droplet, for example, analyte is observed in high relative abundance along the outermost edges, but is of low relative abundance within the interior of the droplet. Upon addition of fructose, however, sample homogeneity improves dramatically while retaining the durability of the dried

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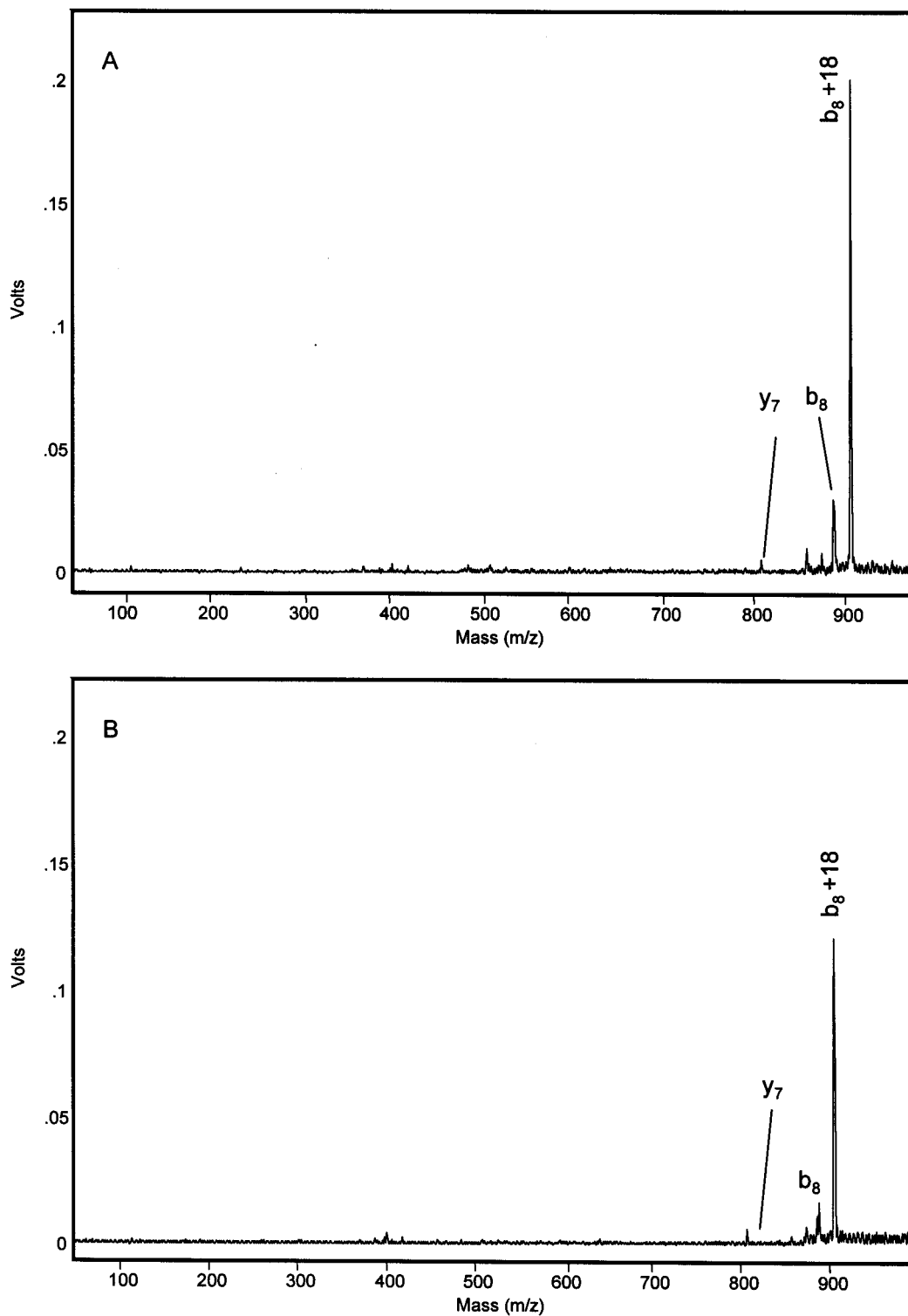


Figure 3. Postsorce decay spectra of bradykinin from an HCCA dried droplet using (A) phenylbutyric acid and (B) fructose as matrix additive.

droplet (allowing several hundred laser pulses without moving the laser). Thus, by using a dried droplet with fructose matrix additive, an optimal balance is struck between durability and reproducibility.

The PSD spectrum of bradykinin $[M + H]^+$ from an HCCA dried droplet is given in Figure 2. Abundant metastable ion fragment signals are present in this spectrum. Although the $b_8 + 18$ ion ($m/z = 904.47$) dominates the spectrum,⁴¹ strong signals

are also obtained for a number of b- and y-type ions. PSD spectra from samples containing the matrix additives phenylbutyric acid (Figure 3A) and fructose (Figure 3B) suggest that the $[M + H]^+$ ions are formed with less internal energy than from a single component HCCA dried droplet (Figure 2). Note that the signal from metastable ion fragments decreases in the order HCCA >

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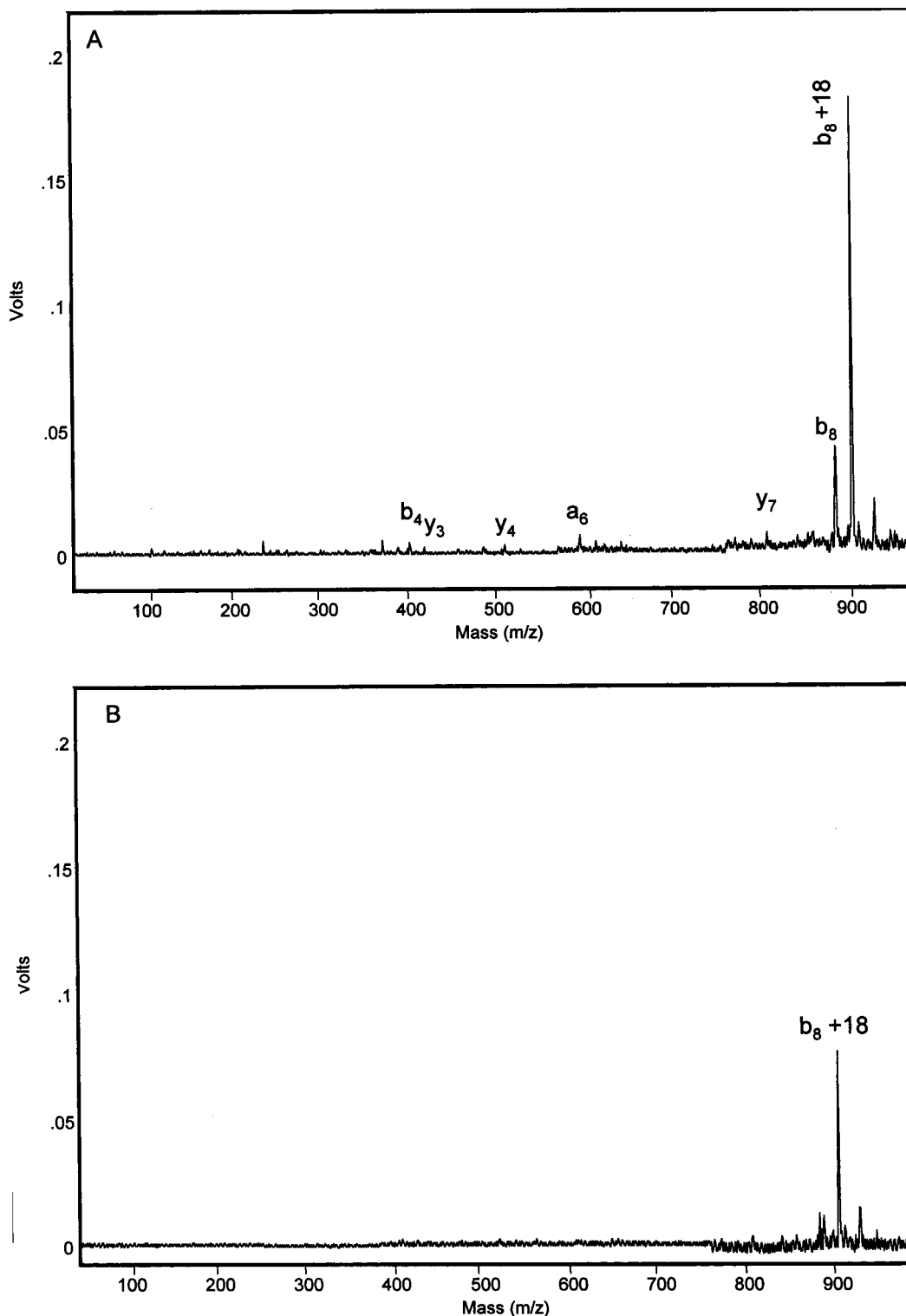


Figure 4. Postsource decay spectrum of bradykinin from (A) HCCA overlayer (B) HCCA/fructose overlayer.

HCCA/PBA > HCCA/fructose. This behavior is illustrated by the relative intensity of the $b_8 + 18$ ion in Figures 2 and 3. Thus, it appears that a smaller portion of the $[M + H]^+$ ions formed from HCCA/fructose and HCCA/phenylbutyric acid dried droplets have sufficient internal energy to dissociate than those from HCCA dried droplets.

Using an overlayer sample preparation further decreases the relative abundance of metastable ion fragments. Figure 4 illustrates

the effect of adding fructose (Figure 4B) to the HCCA overlayer sample (Figure 4A). Note that metastable ion fragment abundance is lower than that for the HCCA/fructose dried droplet (Figure 3B). In fact, other than the $b_8 + 18$ peak and its related ions, no measurable metastable ion fragments are observed in the PSD spectrum when using HCCA/fructose overlayer as a matrix. Similar results were obtained by Beavis and co-workers⁵ and Wilkins and co-workers,^{3,4} who also observed reduced fragmenta-

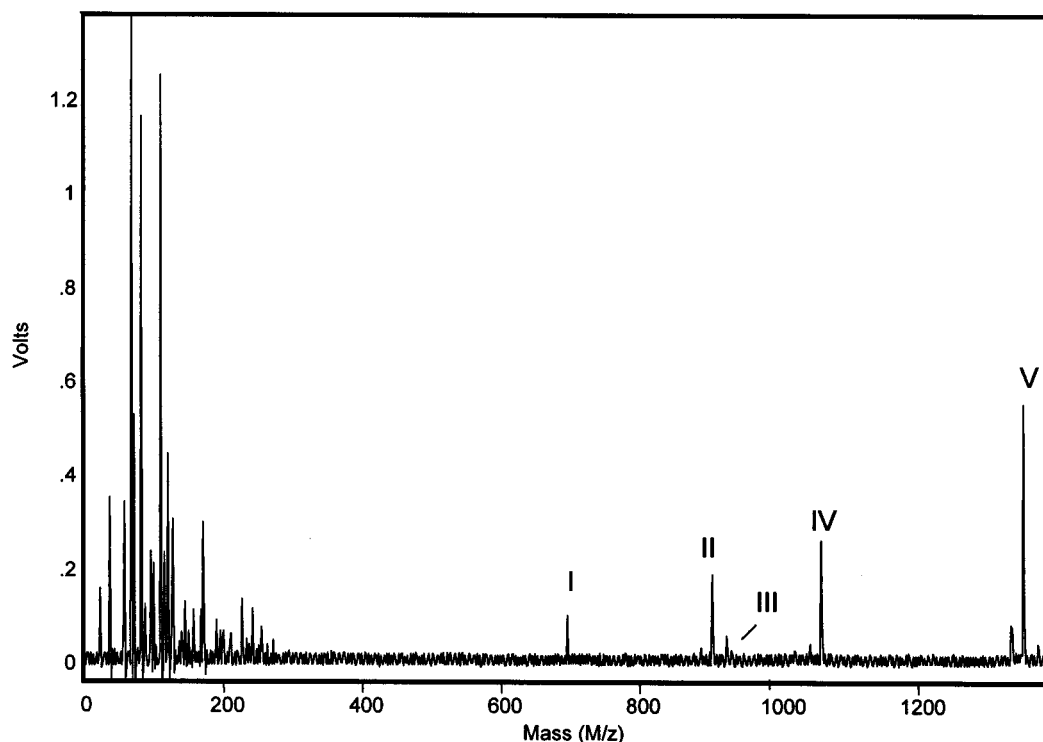


Figure 5. MALDI mass spectrum of a five-peptide mixture taken from an HCCA/fructose dried droplet: (I) splein fragment 32–36 (RKEVY), (II) des-Arg⁹ bradykinin (RPPGFSPF), (III) angiotensin III (RVYIHPF), (IV) bradykinin (RPPGFSPFR), and (V) substance P-amide (RPKPQQFF-GLM-NH₂).

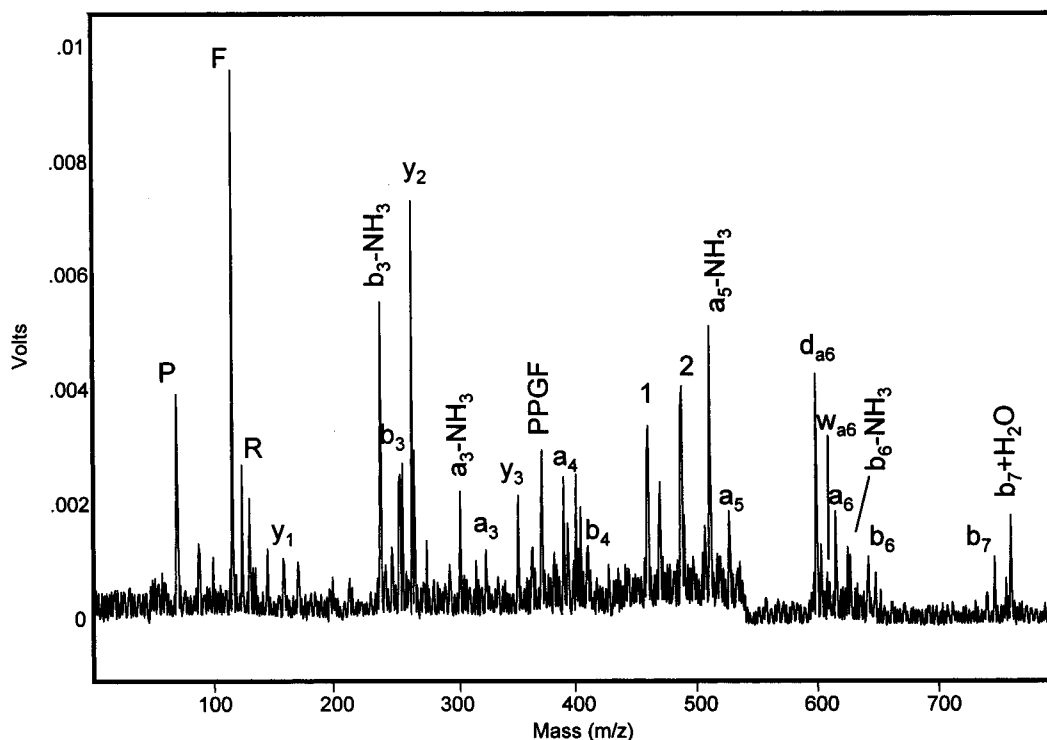


Figure 6. Photofragment ion spectrum of des-Arg⁹ bradykinin (RPPGFSPF) taken from a mixture of five peptides using an HCCA/fructose dried droplet. The fragment ion marked "1" corresponds to the internal cleavage product PGFSP–H₂O or PPFGS–H₂O; the fragment ion marked "2" corresponds to the internal cleavage product PPGFS or PGFSP.

tion using fructose matrixes, and they proposed that pyrolysis of the sugar upon laser irradiation cools the sample, because pyrolysis of the sugar occurs at a lower temperature than pyrolysis

of the peptide. Russell and co-workers⁴² observed that peptides desorbed from matrixes containing sugar additives are detected with greater efficiency by FT-ICR, presumably as a result of the

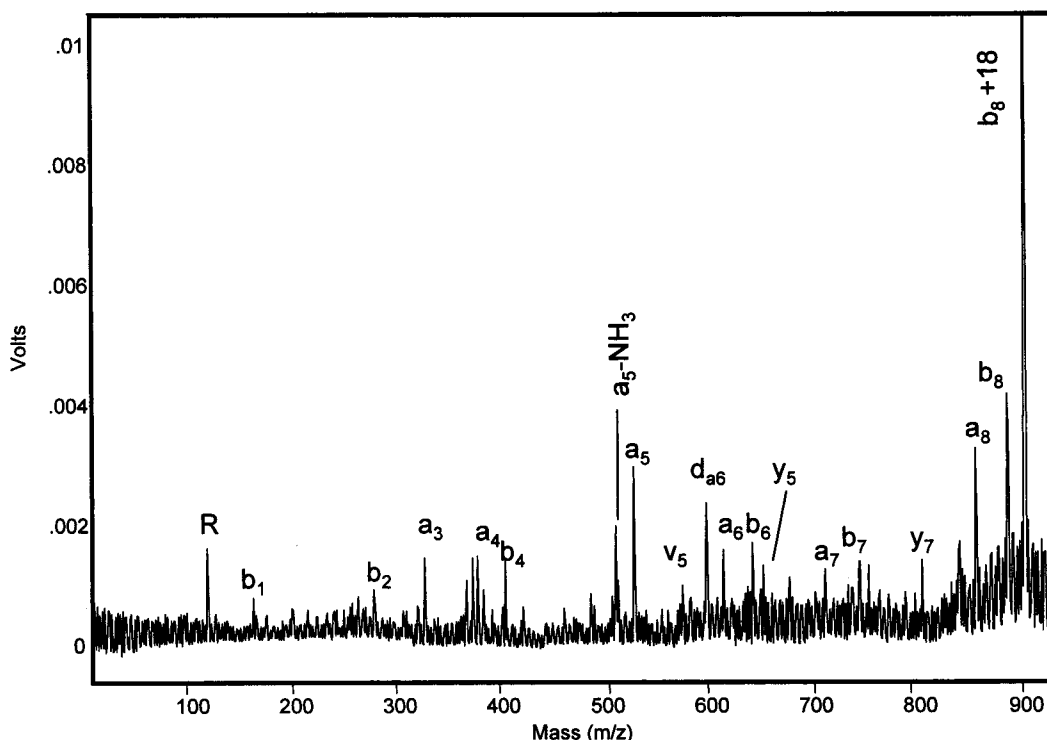


Figure 7. Photofragment ion spectrum of bradykinin (RPPGFSPFR) taken from a mixture of five peptides using an HCCA/fructose dried droplet.

fact that they are formed with less kinetic energy. Whereas the cooling effect of these matrixes can be clearly demonstrated, it is, as yet, incompletely understood.

Photodissociation of Peptides From a Mixture. Figure 5 shows the MALDI-TOF mass spectrum of a five-peptide mixture (10 pmol each of splenin fragment 32–36, angiotensin III, des-Arg⁹ bradykinin, bradykinin, and substance P-amide) taken from a HCCA/fructose dried droplet. It is interesting to note that although equal amounts of each peptide are deposited, the ion yields for each peptide are very different. Such “ion suppression” effects are frequently observed in MALDI and are one area of research in our laboratory.

Figures 6–8 show photofragment ion spectra for des-Arg⁹ bradykinin, bradykinin, and substance P-amide taken from the five-peptide mixture. Note that the improved durability of the fructose matrix additive allowed all of the spectra (representing thousands of laser shots) in Figures 6–8 to be taken from a single sample spot.

The photofragment ion spectrum of des-Arg⁹ bradykinin (Figure 6) contains abundant fragment ions, most of which are the result of amide bond cleavage. In addition to simple backbone cleavage, side-chain cleavage and internal fragments were observed. Side-chain cleavage products are important for resolving isomass residues, such as leucine and isoleucine. The majority of the ions in this spectrum are a- or b-type ions, indicating charge retention on the N-terminus of the peptide as a result of the basic arginine at the N-terminus. The spectrum in Figure 6 contains sufficient information to unambiguously sequence the peptide (for clarity, not all peaks are labeled). Although significant numbers

of immonium ions (those for proline, phenylalanine, and arginine) are observed in this spectrum, it is not deficient in sequence informative data. The peaks labeled 1 and 2 correspond to internal cleavage ions. Although at first glance, internal cleavage ions might seem to complicate the spectrum, they can be very useful in reconstructing peptide sequence, often providing confirmation of sequence data from backbone cleavage products or providing additional sequence data where an a-, b-, or y-series may be lacking. Note that no x-, z-, or c-type ions are marked in this spectrum. Although the occasional x-, z-, or c-ion may be observed, they are infrequently encountered in photofragment TOF spectra and are, thus, of little analytical utility.

Similar fragmentation is observed in the photofragment ion spectrum of bradykinin (Figure 7). The photofragment ion spectrum of bradykinin contains fragment ions that exhibit both N- and C-terminal charge retention, because the peptide contains arginine at both the N- and C-termini. However, Schnier and co-workers showed that N-terminal cleavages dominate the fragment ion spectrum of bradykinin, because the C-terminal arginine exists as a gas-phase zwitterion, which reduces its basicity.⁴³ The N-terminal arginine then becomes the most basic residue in the molecule, thus explaining the predominance of N-terminal charge retention in the fragment ion spectrum. Again, several side-chain cleavages are observed in the photofragment spectrum, particularly the d_{a6} fragment. Once again, the photofragment ion spectrum contains enough data to unambiguously extract the sequence of this peptide.

Photodissociation of “hot” bradykinin $[M + H]^+$ ions from a conventional HCCA dried droplet provided additional interesting

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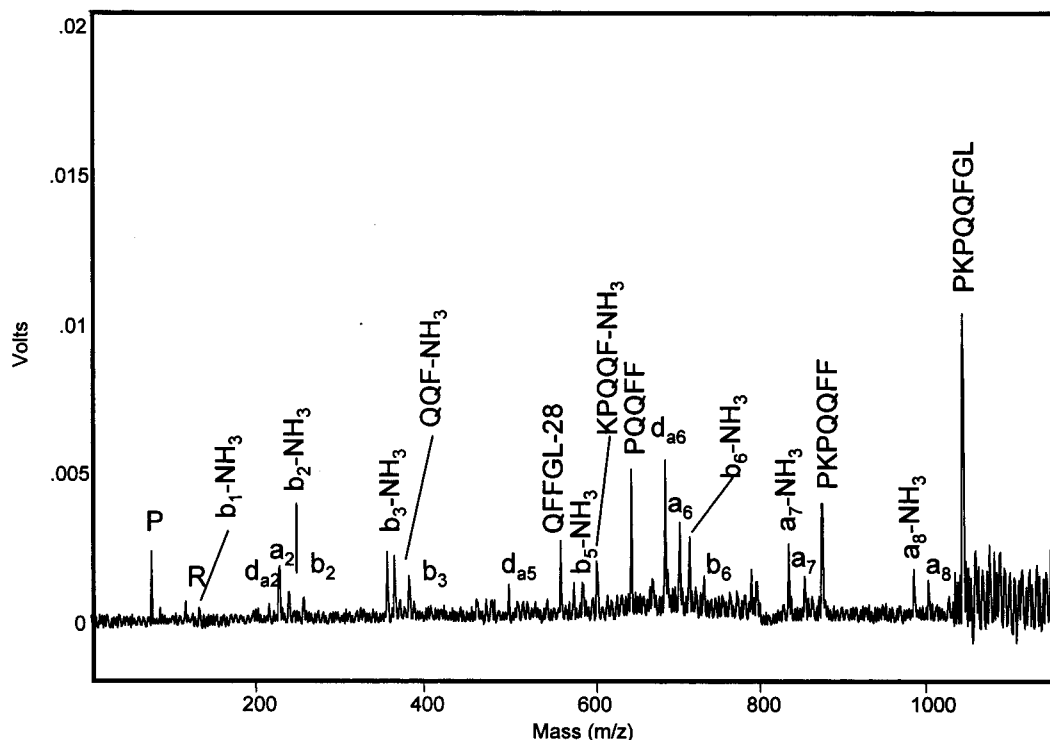


Figure 8. Photofragment ion spectrum of substance P-amide (RPKPQQFFGLM-NH₂) taken from a mixture of five peptides using an HCCA/fructose dried droplet.

results. In this case, >60% of the fragment ion current is carried by fragment ions below m/z 200 (e.g. immonium ions). Although these fragments are informative from the standpoint of amino acid analysis, they do not provide sequence information. In contrast, these same low-mass-fragment ions carry <2% of the total fragment ion current in the photofragment ion spectrum taken from the optimized sample preparation method. By minimizing the contribution to the photofragment ion spectrum of these low mass ions, the sequence information content of the spectrum is maximized.

In the photofragment ion spectrum of substance P-amide (Figure 8), a- and b-type ions are observed, indicative of N-terminal charge retention. Substance P has a basic arginine at position 1 and a lysine at position 3. Thus, the charge primarily resides on the N-terminal fragments. Once again, a number of side chain and internal cleavage products are observed in the photofragment ion spectrum. Additionally, significant amounts of $F_i^+-NH_3$ ions are observed in this spectrum. This is a fragment ion pathway of higher energy than simple bond cleavage and is occasionally observed in photofragment ion spectra.

CONCLUSIONS

Fructose and phenylbutyric acid are suitable matrix additives both to reduce background from postsource decay and to improve overall reproducibility in the MALDI event. We conclude that the postsource decay signals may be minimized by using an overlayer sample preparation and by the addition of fructose or PBA. Unfortunately, the overlayer sample preparation is ablated much more quickly than is the dried-droplet preparation, requiring more sample to generate a photofragment ion spectrum. The HCCA/fructose matrix is an excellent choice for photodissociation studies, because it produces the lowest PSD background signal while maintaining acceptable short-term precision.

By taking advantage of the postsource decay-suppression effect inherent to peptide mixtures, peptide $[M + H]^+$ ions were formed from the HCCA/fructose dried droplet with very low internal energies. Fragmentation can be induced via photodissociation, allowing sequence information to be determined. In addition to backbone cleavages, extensive side chain and internal cleavages are observed, generating more structurally informative information than conventional postsource decay analysis.

Future studies include photodissociation at different wavelengths. In particular, it will be interesting to investigate how the photofragment spectrum changes if 248-nm (KrF excimer) laser irradiation is used. Improvements in pulse characteristics (narrower pulse width) will extend the current capabilities of the method by increasing MS¹ resolution. A pulse width on the order of 2–3 ns would allow the selective photodissociation of one peak out of the middle of the isotopic distribution, an experiment that would prove valuable in a number of instances, such as determination of a site of deuteration.

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