

Effects of the Water Content in the Sample Preparation for MALDI on the Mass Spectra

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Ion abundances in the MALDI TOF mass spectra of the model peptides (bradykinin, α -melanocyte stimulating hormone, and melittin) change significantly as water is added to the solution used for dried droplet sample preparation. Changes in the surface tension of the solution can be invoked to explain some of the observed effects on the mass spectra. For example, at low surface tensions, the solution droplet spreads over a larger surface area, resulting in a dilution effect and thereby lowering the $[M + H]^+$ ion yields. Analyte ion yields also have a dependence on matrix crystal morphology. The faster drying samples (low water percentage) promote better inclusion of the analyte into the matrix crystals as compared with the slower drying samples (high water percentage). More efficient inclusion of the analyte in the matrix crystal leads to a better matrix–analyte interaction and hence to higher $[M + H]^+$ ion yields. We present new data that suggest that analyte conformation also influences the MALDI ion yields. The suggestion of conformation affecting MALDI ion yields is based on solvent composition dependence for MALDI H/D exchange data and circular dichroism spectra.

Matrix-assisted laser desorption/ionization (MALDI) is one of the most widely used techniques to produce intact gas-phase ions of high-molecular-weight biomolecules for subsequent mass spectrometric analysis. It is generally agreed among MALDI practitioners that the quality of the mass spectral data is dependent on certain factors related to sample preparation, e.g., (i) sample preparation method employed, (ii) selection of the matrix compound, (iii) presence of detergents and/or buffers, (iv) pH of the solution, (v) analyte characteristics, and (vi) solvent or combination of solvents.

Although MALDI is a very popular ionization technique, the processes that lead to desorption and ion formation are not well understood. In an effort to further understand the interactions between the matrix and the analyte, several groups have examined the morphology of the sample spots.^{1–9} The MALDI sample

morphology obtained by the so-called dried droplet sample preparation has been studied with the use of several microscopic techniques such as optical microscopy¹ and scanning electron microscopy.^{4,5} Doctycz et al.¹ used optical microscopy to examine the morphology of sample spots for three matrixes. They reported that, in the case of the matrixes used, the samples appeared to be heterogeneous and made up of two phases, a crystalline birefringent matrix phase and an isotropic phase containing the analyte. Dai et al.² examined the analyte distribution in MALDI samples prepared by utilizing several sample preparation techniques with laser confocal fluorescence microscopy. The results from these studies suggest that MALDI deposits prepared using the dried droplet method are composed of different kinds of crystals, and the sample morphology depends on the identity of the matrix. These studies fail to consider the effects of the sample solution conditions, such as solvent, on the morphology of the spots or the mass spectra. Cohen and Chait¹⁰ were the first to consider the influence of matrix solution conditions on the MALDI mass spectra. Their results showed a mass discrimination effect on the MALDI mass spectra of a mixture of peptides that depends on the solvent composition and pH of the sample solution as well as on the rate of crystal growth on the ion yields. Börnsen et al.¹¹ have also investigated the influence of solvents and detergents on the MALDI mass spectra. They evaluated the ion yields of three proteins as a function of different solvent–water (1:1) mixtures and concluded that an equal volume mixture of water and 2-propanol produced the best quality spectra.

Our experiments are designed to provide new information on solvent effects on the MALDI samples and consequently on the mass spectral data. Methanol and α -cyano-4-hydroxycinnamic acid is the solvent–matrix combination chosen for these experiments because this combination is the most frequently used for MALDI. We observe dramatic effects on the MALDI peptide ion yields as a function of the water content in the sample solution for dried

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droplet sample preparation. Because solvent can affect the conformation of biomolecules, we have used H/D exchange chemistry to probe the conformation of the model peptides used for this study. The conformational changes proposed on the basis of mass spectrometry H/D exchange studies are compared with data obtained by using circular dichroism (CD) spectroscopy to test whether conformation affects the ion yields of the model peptides chosen for this study. Our results are discussed in terms of the implications that these peptide conformational changes have on the MALDI ion yields.

EXPERIMENTAL SECTION

MALDI Spectra. The MALDI TOF mass spectra were obtained using a PerSeptive Biosystems Voyager Elite XL TOF mass spectrometer equipped with a pulsed nitrogen laser emitting at 337 nm. All spectra were acquired in a positive ion mode, and 200 laser shots were averaged for each spectrum. Spectra for two of the peptides used in this study, bradykinin and α -melanocyte stimulating hormone (α -msh), were acquired using the delayed extraction linear mode for all the experiments. These instrumental conditions provided unit mass resolution for bradykinin and α -msh. For melittin, the data were acquired in the delayed extraction reflected mode in order to obtain unit mass resolution. For the water addition MALDI experiment, unit mass resolution for melittin was not required; therefore, in that experiment the instrument was utilized in the delayed-extraction linear mode like for the other two peptides. The acceleration voltage used for the linear mode was 25 kV, with the grid voltage set at 96.5%.¹² The delay times used were 100, 150 and 250 ns for bradykinin, α -msh, and melittin, respectively. The parameters for the reflected mode were 25 kV acceleration voltage, 75% grid voltage, and a delay of 200 ns. These conditions generated isotopic resolution spectra for all the peptides used in this study with the exception of melittin in the delayed extraction linear mode.

Sample Preparation for the Water Addition MALDI Experiments. The samples were prepared for MALDI analysis using the dried droplet method. The matrix used in these studies was α -cyano-4-hydroxycinnamic acid (HCCA, Aldrich). The low water content solutions were prepared inside a drybox filled with nitrogen. Stock solutions of matrix dissolved in methanol and the peptides bradykinin, α -msh, and melittin (Sigma) dissolved in deionized water (Milli-Q Water System, Millipore) were prepared. Aliquots from these stock solutions were diluted so as to obtain a final sample solution containing 1 pmol of peptide and a matrix-to-analyte molar ratio of 3000:1. The aliquots were diluted with the required amount of deionized water and anhydrous methanol (99.8%, Aldrich) to get the desired water percentages (v/v) in solution. Several 1- μ L droplets were deposited onto the stainless steel sample plate and allowed to air-dry before introduction into the instrument. Five mass spectra were collected for each water percentage sample at constant laser power. The average integrated area of the $[M + H]^+$ peak was plotted against the water percentage. The error bars were calculated using the standard deviation of the average areas.

Sample Preparation for MALDI Hydrogen/Deuterium Exchange Experiment. All the solutions for the MALDI H/D

exchange studies were prepared inside a nitrogen-filled drybox. The relative humidity inside the drybox was maintained below 7%. Stock solutions of HCCA dissolved in MeOD- d_4 (99.9%, Cambridge Isotopes Laboratories, Inc.) and the peptides dissolved in D₂O (Aldrich, 99.9%, and Cambridge Isotopes Laboratories, Inc., 99.8%) were prepared. Aliquots from these stock solutions were diluted to give a 3000:1 matrix-to-analyte ratio and a final analyte concentration in the micromolar range. The stock solutions were diluted with MeOD- d_4 and D₂O in specific proportions to obtain the desired amount of water in solution (% v/v). These solutions were incubated for a period of 24 h before analysis. Various 1- μ L sample droplets were deposited on top of the stainless steel sample plate and allowed to dry inside the drybox to avoid back-exchange with the ambient water. In an attempt to avoid back-exchange during sample transportation to the instrument, the dried sample was placed inside a Ziploc (Dow Brands L.P.) freezer bag back-filled with dry nitrogen. The Ziploc freezer bag containing the sample was then placed inside an Atmosbag (Aldrich), continuously purged with nitrogen, and taped around the instrument sample inlet. Five spectra were acquired for each water content sample solution at a constant laser power. The areas for each one of the isotopic peaks were divided by the sum of the areas for all the peaks in the distribution (weighted areas). The centroid of the isotopic peak distribution was calculated by adding all the weighted areas corresponding to each mass in the distribution. The average calculated centroid of the isotopic peak distribution was plotted against the percentage of water in the solution. The error bars in the plots represent the standard deviation of the average centroids of the peak distributions.

Circular Dichroism Experiments. The circular dichroism (CD) spectra were acquired by using an AVIV 62DS circular dichroism spectrometer at 25 °C. Calibration of the instrument's detection system was performed by measuring the (1S)-(+)-10-camphorsulfonic acid (CSA) ellipticity ratio of the 290-nm to 192.5-nm absorption bands. This measured ratio was between 1.9 and 2.2, which is the accepted range of the instrument for all the experiments. Stock solutions of bradykinin, α -msh, and melittin dissolved in deionized water were prepared. Solutions containing 10–100% water in methanol and peptide were prepared from the stock solutions for each peptide. The final peptide concentration was 1×10^{-4} M for all the solutions. The data reported herein are the averages of two spectra that were averaged, smoothed, and plotted using KaleidaGraph 3.0 (Synergy Software) after converting ellipticity into molar ellipticity.

RESULTS

$[M + H]^+$ Ion Abundances. In an effort to investigate the effect of the analyte conformation on MALDI, we report the $[M + H]^+$ ion yields as a function of the water added to the solution. Figure 1 shows histograms for the average area of the $[M + H]^+$ ion peak versus the water content in the sample solution of the peptides. In the case of bradykinin (Figure 1a), the average $[M + H]^+$ peak area increases as water is added until it reaches a maximum (30% water) and then decreases. All three of the peptides show similar results. At low water contents, the $[M + H]^+$ ion abundances are small for the three peptides studied. Moreover, as water is added, the $[M + H]^+$ ion yields reach a maximum. The maximum ion yields occur at 20% and 40% water for melittin and α -msh, respectively. As more water is added to

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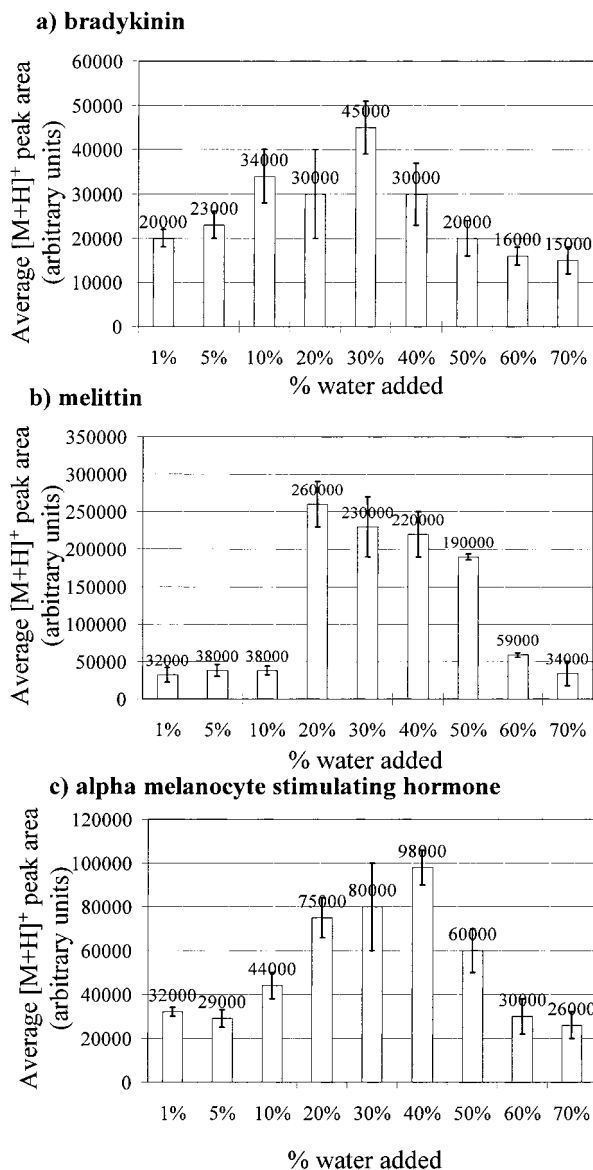


Figure 1. Plots of the average $[M + H]^+$ peak area versus the water added to a methanolic solution of the peptides (a) bradykinin, (b) melittin, and (c) α -melanocyte stimulating hormone. The error bars on the plots correspond to the standard deviation of the average $[M + H]^+$ peak areas of five different spectra.

the sample solution, the ion abundances decrease. The relative abundances of the Na^+ and K^+ adduct peaks with respect to the $[M + H]^+$ decrease as water is added to the solution (Figure 2).

MALDI H/D Exchange Experiments. As the solvent environment is altered, we observe changes in the extent of H/D exchange, which we interpret in terms of peptide conformational changes. Changes in peptide conformation could be one of several factors responsible for the effects observed on the $[M + H]^+$ ion yields. H/D exchange is frequently used to probe peptide conformational changes;^{13–17} consequently, we used this method

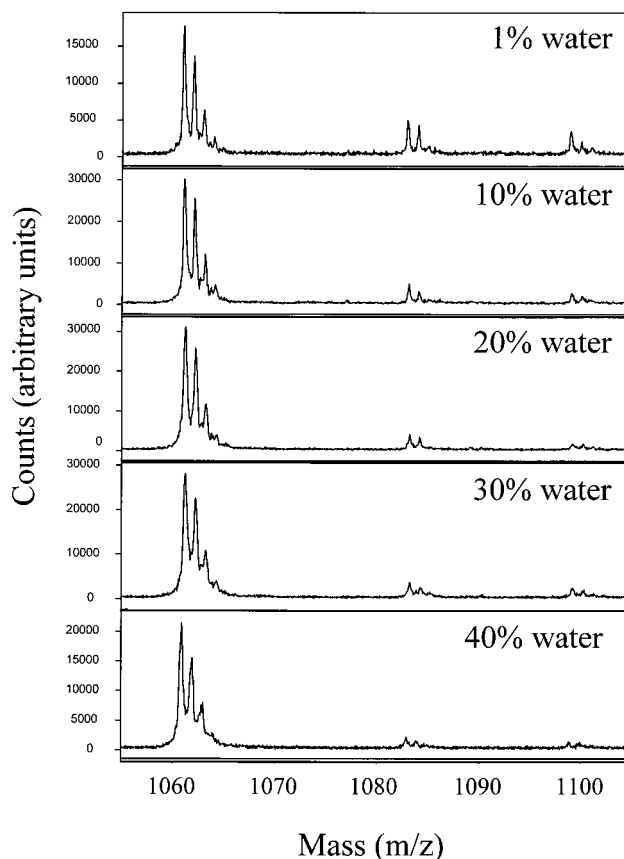


Figure 2. MALDI mass spectra of bradykinin as water is added to a methanolic solution. The matrix used was α -cyano-4-hydroxycinnamic acid. Note that as the water content in solution is increased the relative abundances of the $[M + \text{Na}]^+$, $[M + \text{K}]^+$ adduct ions decreases with respect to the $[M + H]^+$.

to assist in explaining the observed effects on the mass spectra as D_2O is added to the sample solution. Results from these experiments for the three peptides in this study are shown in Figure 3. All the solutions used in these experiments were prepared using deuterated solvents. Then, it is reasonable to assume that the protonated species in mass spectrum is likely to be the $[M + \text{D}]^+$ ion rather than the $[M + H]^+$ ion. To calculate the exchange percentages for the peptides under study, it was necessary to correct for this assumption. For example, in the bradykinin MALDI H/D exchange experiment in Figure 3a, the corresponding exchange percentage for the 1% D_2O solution was calculated using the following mathematical expression:

$$\% \text{ exchange} = \frac{(1074.8 - 1060.57) - 1}{17} \times 100 = 77.8\% \quad (1)$$

where 1074.8 Da is the average isotopic peak distribution centroid for the 1% D_2O sample solution, 1060.57 Da is the monoisotopic mass of bradykinin $[M + H]^+$ ion, and 17 is the number of exchangeable hydrogens on the bradykinin molecule (5 amide hydrogens on the backbone and 12 on the side chains and termini). The subtraction of 1 mass unit is due to the mass difference between the two isotopes of hydrogen and corresponds to the correction needed if the protonated species is taken to be $[M + \text{D}]^+$ rather than $[M + H]^+$. The exchange percentages for

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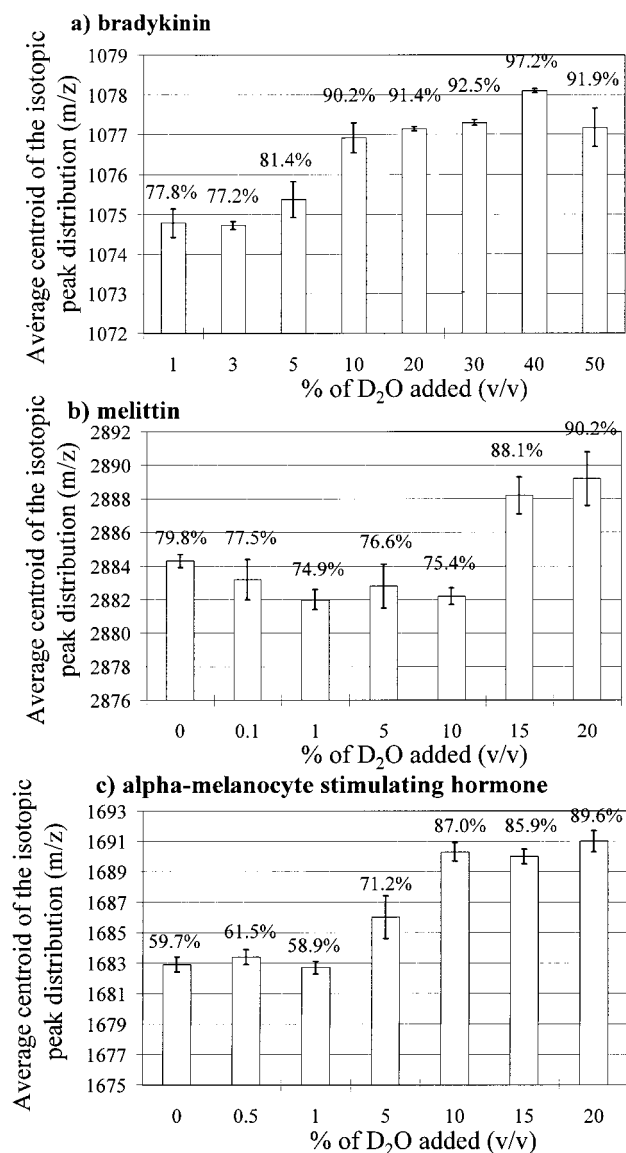


Figure 3. Plots of the average centroid of the isotopic peak distribution versus the amount of D₂O added to a MeOD-*d*₄ solution of the peptides (a) bradykinin, (b) melittin, and (c) α -melanocyte stimulating hormone. The error bars correspond to the standard deviation of the average centroid calculated for five different spectra, and the numbers on top of the bars are the calculated exchange percentages.

all the solutions were calculated the same way as for bradykinin. Monoisotopic $[M + H]^+$ ion masses of 1665.79 and 2845.76 Da and numbers of exchangeable hydrogens of 27 and 47 for α -msh and melittin, respectively, were used to calculate their exchange percentages.

The results for bradykinin (Figure 3a) show that, at low D₂O percentages, the exchange percentage is low. As the water content in solution is increased, the exchange percentage increases until it reaches almost 100% exchange. For the solutions containing 1% and 3% D₂O, the exchange percentage is approximately 77% and constant (within experimental error). The 77% exchange corresponds to exchange of 14 out of the 17 labile hydrogens, leaving three hydrogens protected from exchange. As the D₂O content in solution is increased, the exchange percentage increases to a maximum of 97.2% at 40% D₂O, corresponding to

an average of approximately 16.5 hydrogens exchanged. The results for α -msh and melittin (Figure 3b,c) also show trends in exchange percentages similar to those for bradykinin. At low D₂O contents, the exchange percentage is lower for all the peptides; as water is added to the solution, the exchange percentages increase, denoting a change in peptide conformation due to the variable solvent environment.

CD Spectra. Circular dichroism is a very popular technique for monitoring peptide conformational changes in solution. The technique serves as a diagnostic tool to monitor possible changes in conformation of the peptides as the solvent environment is changed from methanol to an aqueous environment. The CD spectra for the three peptides used in this study are shown in Figure 3. The CD spectra of bradykinin (Figure 4a) in all the solvent compositions are composed of three characteristic peaks: an intense trough around 204 nm, a weak trough around 234 nm, and a positive peak at 222 nm. As the water content in the bradykinin solution is increased, the 204-nm trough intensifies. These spectra resemble the typical CD spectra for bradykinin published previously.^{18,19}

The CD spectra for melittin in 1:10 water/methanol solution resemble the spectrum of a peptide in alpha helical conformation²⁰ (Figure 4b). It shows a double minimum at 207 and 222 nm and a strong maximum close to 190 nm. As the water content of the solution is increased, the CD spectrum changes gradually toward the typical spectrum of a disordered peptide, showing a characteristic negative peak at 200 nm.²⁰ Melittin is known to adopt mainly a helical conformation in methanolic solution²¹ and a random conformation in deionized water.²²

The CD spectrum for α -msh in 1:10 water/methanol solution shows a negative peak at 208 nm and a small maximum at 227 nm (Figure 4c). Going from the 10% water to the 30% water solution, the 208-nm peak loses intensity and shifts to shorter wavelengths. The 40% and 50% spectra show that the negative peak intensifies and also shifts slightly to shorter wavelengths as compared to the 30% water spectrum. For the next three solutions (70%, 80%, and 100%), the negative peak intensifies and continues shifting to shorter wavelengths in a more pronounced way than for the 40% and 50% water solutions. The maximum around 225 nm also intensifies and shifts to shorter wavelengths as the water content is increased.

DISCUSSION

The discussion of variations in the $[M + H]^+$ ion yields as a function of water content in the sample solution (Figure 2) will focus on (1) morphology effects and (2) peptide conformational changes. Among the morphology effects, there are several factors to be considered: (i) how the matrix-analyte interaction in solution affects the distribution of species in the crystal lattice, (ii) effects of analyte solvation in the crystal lattice, and (iii) overall

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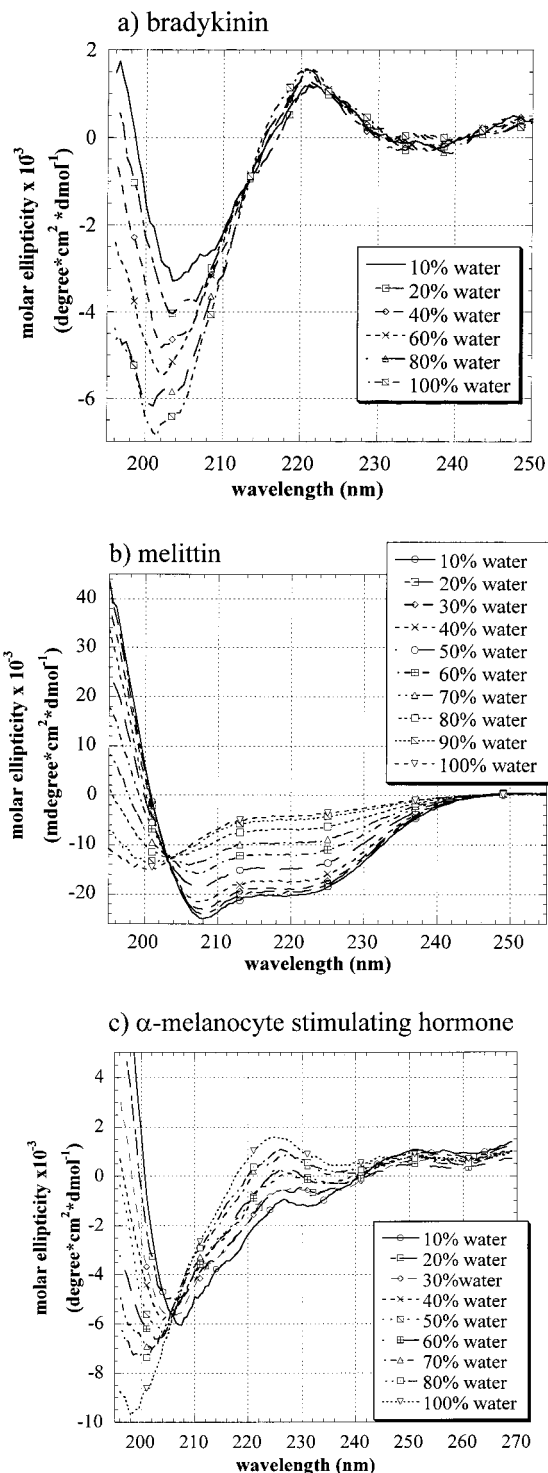
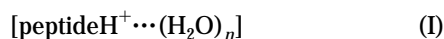


Figure 4. Solution-phase circular dichroism spectra for the three peptides (a) bradykinin, (b) melittin, and (c) α -melanocyte stimulating hormone with different ratios of water and methanol. Note that the conformation of each of the three peptides is changing as the water content in the solution is increased.

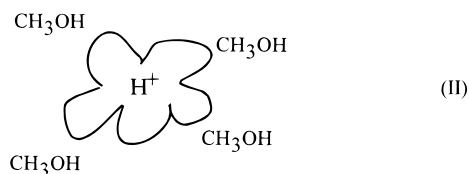
morphology of the matrix and analyte crystals. Our data support the notion that variations in surface tension of the solvent along with peptide conformational changes as water is added to the sample solution are responsible for the effects observed. Clearly, the presence of matrix in solution could affect the conformation of the peptides; however, the only variable considered is the solvent. Similarly, the pH of the solution is a concern, and studies

are being done to assess the effect of pH on the H/D exchange experiments.

The interaction of the matrix and the analyte in solution before the crystals are formed on the probe by evaporation of the solvent influences the ion yields. Cohen and Chait¹⁰ proposed a reversed-phase-like partitioning of the peptide between the growing matrix crystal and the bulk solution during the crystallization process that causes discrimination against the low-mass components in the MALDI mass spectra. Herein we expand upon their general hypothesis to encompass solvent effects on MALDI ion yields. It is generally known from principles of chromatography that analyte partitioning between liquid and solid phases is a function of several parameters, including the solvent. As the water content in the sample solution is increased, the polarity of the solvent also increases, causing the analyte to partition between two increasingly different phases. In separate papers, Cohen et al. and Beavis et al. argue that the analyte interacts with the nonpolar face of the growing crystal.^{10,23} Thus, the hydrophobicity of the peptide determines the extent of interaction with the matrix crystal and hence the incorporation of the analyte into the crystal lattice. Owing to the hydrogen bond donor–acceptor capabilities of many matrix compounds and the hydrophilicities of acidic/basic amino acid side chains, it is also important to evaluate polar matrix–analyte interactions. Peptides are generally basic and exist in their protonated (hydrophilic) form in solution. Thus, solvation of the charge site plays an important role in the conformation.



In the binary solvent system used, methanol and water, the latter is more basic. In solutions containing small amounts of H_2O , the water molecules solvate the charge site as illustrated in (I). If there is very little or no water in solution, the peptide “coils” to intramolecularly solvate the proton (II).



This form of the peptide is more hydrophobic because less polar regions of the molecule interact with the solvent. This “coiled” form of the molecule interacts more with nonpolar solvents and possibly with less polar matrixes. Another important issue is how solubility of the different peptide forms, I and II, affects inclusion of the analyte in the matrix crystals. These effects will be studied in detail in future experiments.

One of the more notable effects of solvent we have observed are the relative abundances of $[\text{M} + \text{Na}]^+$ and $[\text{M} + \text{K}]^+$ ions. For example, the relative abundances of Na^+ and K^+ adduct ion peaks decrease as the water content is increased (Figure 2). We interpret this in terms of a competition between water and the peptide for the Na^+ and K^+ ions. That is, the Na^+ and K^+ ion affinities for the peptide are greater than those for methanol, whereas the Na^+ and K^+ ion affinities for H_2O are greater than

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those of the peptide.^{24–26} Thus, in the absence of water, Na⁺ and K⁺ are “solvated” by the peptide. However, as water is added, these ions are solvated by the more polar solvent.

The results from studies examining the effect of trace amounts of water on MALDI ion yields clearly illustrate that the presence of water increases the ion abundances. The residual solvent could be stabilizing or destabilizing interactions between the matrix and the analyte or even serving as hydrogen bond bridges between these two molecules. Water has a higher capacity for hydrogen bonding than methanol, and also the acidity of the matrix changes as the water content in solution is increased. There are conflicting views on the role of matrix acidity (basicity) in MALDI.^{27,28} Therefore, it is not possible, at this juncture, to delineate all factors related to the influence of solvent on matrix acidity. Independent of solvent-related acidity issues, it is reasonable that, as the molar fraction of water in the solvent system is increased, the interaction between the analyte and the matrix may change. In the case of the matrix and model peptides used for this study, the matrix–analyte interaction is enhanced as water is added. This will ultimately lead to higher [M + H]⁺ ion yields, even though in our case this is not the dominating factor in the ion yields for the higher water content solutions. For other peptides and matrix compounds, the matrix–analyte interaction might be weakened.

The changes in surface tension as water is added to the solution affect the morphology of the matrix crystal as well as the crystal size. In addition, the low surface tension of the solutions that contain less than 20% water results in spreading of the sample droplet over a larger area, a dilution effect, as it is placed on the stainless steel sample probe to dry. The surface tension of the solution also affects the rate of solvent evaporation. Droplets that contain a low percentage of water evaporate more quickly than do the samples that contain a higher water percentage. Fast drying results in formation of smaller crystals and entrapment of the analyte molecules into the matrix crystals.^{10,29} We propose that better entrapment of the analyte into the crystal lattice can be due to at least two possibilities: (i) rapid evaporation, on average, yields smaller crystals that provide more surface area for the attachment of the analyte to the surface of the crystal and possibly less selective attachment of the analyte and/or (ii) there is an increase in the inclusion of impurities (analyte) in the matrix crystal lattice. This is consistent with the idea that better analyte entrapment improves the interaction between the matrix and analyte and that the observed results in the mass spectra are higher ion yields.

Fast evaporation and crystallization might be responsible for another factor influencing the [M + H]⁺ ion yields. The rapidly formed crystals tend to trap solvent and other impurities in the pockets of the lattice.²⁹ This physical entrapment of residual solvent molecules could influence the ion yields. It is reasonable to think that the principal factor responsible for the decrease in

the [M + H]⁺ ion yields for the higher water content solutions is the weakening of the matrix–analyte interactions. The weakening of the matrix–analyte interaction is due to the exclusion of the analyte from the matrix crystal during the crystal growth process. This is facilitated by better solvation of the analyte by the increasing amount of water in solution. The increase in water content stabilizes form I of the peptide, which can be classified as a preformed ion. According to the preformed ion model,^{30–32} the ion yields increase if the analyte molecule is ionized before the desorption and ionization processes take place in the ion source. This concept does not seem favorable in our experiments, due to the fact that we do not observe a constant increase in the ion yields with water addition. This is probably because peptide form I is excluded from the growing matrix crystal lattice in the reversed-phase-like partitioning mechanism proposed by Cohen and Chait.¹⁰

An interplay among all the factors mentioned above is responsible for the observed effect in the mass spectra with the addition of water to the sample solution. The results presented in Figure 1 show that for all the peptides, the trend in [M + H]⁺ ion abundances is similar. At low water contents, the overall abundances of the [M + H]⁺ ions are low. Note also that the yields of [M + Na]⁺ and [M + K]⁺ ions decrease relative to the [M + H]⁺ ion as the percentage of water increases. As the water content is increased, the [M + H]⁺ ion abundance increases and reaches a maximum. Since the solvent system, sample probe, and the peptide and matrix concentrations were the same for the three peptides, we would not expect peptides to crystallize in a different fashion. The morphology of the samples should then be very similar for the sample solutions containing the same water percentage, regardless of the identity of the peptide and provided that their hydrophobicities are similar. We suggest that the dominant factor controlling the ion yields in the low and high water content solutions is the effect of sample morphology. The fact that the effect of sample morphology on the [M + H]⁺ ion yields is not the same for each peptide suggests that other factors influence the ion yields. Peptide conformational changes are a reasonable explanation for such effects. For example, an equilibrium between conformers such as I and II would be strongly dependent on solvent. That is, as the solvent environment is made increasingly aqueous, form I is favored. Peptide conformational changes influence the accessibility of the peptide's protonation sites for MALDI. Furthermore, it is possible that different conformations of the peptide interact differently with the matrix molecules.

Due to its high dielectric constant, water has a higher capacity to stabilize dipoles and charges compared to polar organic solvents such as methanol. Consequently, peptides dissolved in organic solvents cannot hydrogen bond with the solvent, as is common for peptides dissolved in water. Nonpolar solvents promote intramolecular hydrogen bonding in peptides. Therefore, it would not be surprising if the model peptides used in this study acquire more tightly folded conformations in methanol as compared to water. The results from the H/D exchange experiments in

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methanol and water solution provide clear evidence of conformational changes, and these experiments are consistent with the previously reported data on these peptides. For example, Marlborough et al.¹⁸ reported CD data for bradykinin that suggest it adopts a more folded or compact conformation in organic solvent. The conformation of melittin in methanolic solution has been determined from ¹H NMR nuclear Overhauser enhancement data using distance geometry and restrained molecular dynamic analyses.²¹ The structure of melittin in methanol consists of two α -helical regions joined by a "hinge" between residues 11–12,²¹ whereas in deionized water, melittin assumes a conformation that is mostly random coil.²² Our H/D exchange experiments for bradykinin in methanol show that 3 of the 17 exchangeable hydrogens are inaccessible and exchange slowly. This result is surprising because bradykinin is a small peptide (nine residues), believed to have little secondary structure.^{19,33} The exchange percentages increase as D₂O is added to the solution, which clearly indicates that the conformation of bradykinin is changing when going from methanol to water/methanol (20%:80%) solvent. The changes in conformation of bradykinin for different water solutions are also evident in the CD spectra (Figure 4a).

The results acquired for melittin and α -msh resemble those obtained for bradykinin. The conformation of melittin in methanolic solution protects some labile hydrogens from exchange. These data are consistent with the helical structure that melittin assumes in methanol.²¹ The exposure of more labile hydrogens for exchange as water is added to the solution is also consistent with the random conformation adopted by melittin dissolved in water with low concentration of dissolved salts.²² These conformational changes also are evidenced in the CD spectral data for melittin (Figure 4b). Although no previous information on the conformation of α -msh in methanol is available, it appears that α -msh acquires some secondary structure when dissolved in methanol. α -msh adopts a conformation in methanol solution that protects approximately 40% of its labile hydrogens from exchange with the surrounding solvent. As the water content of the solvent is increased, more hydrogens become exposed to solvent. This change in α -msh conformation from some kind of organized secondary structure element to a less ordered conformation is confirmed by the CD spectral data (Figure 4c). In general, it

seems that peptides dissolved in methanol acquire a folded conformation (II) that does not allow all the labile hydrogens to exchange. This might not be the case for other peptides and/or proteins. The unfolding of all peptides provides better accessibility for the matrix and solvent molecules to reach the possible protonation sites. If the protonation sites become more available, then the observed effect should be to increase the $[M + H]^+$ ion yields.

CONCLUSIONS

Our results show that the $[M + H]^+$ ion yields are strongly dependent on the amount of water added to the sample solution for dried droplet sample preparation. As water is added to the solution, we conclude that changes in the surface tension alter the morphology of the sample spots and hence are partially responsible for the observed effects. Furthermore, the matrix–analyte interactions in solution prior to crystallization appear to play a very important role in the ionization process. We further speculate that conformational changes of the biomolecules occur as water is added to the solution. Results from H/D exchange mass spectrometric experiments as well as with the data obtained from circular dichroism experiments are consistent with this interpretation of the observed effects. Additional studies are underway to study the effects of solvent in an effort to understand the role of matrix–analyte interaction on the MALDI process; however, the study clearly illustrates that, with careful selection of the water content in the sample solution, the limits of detection of the MALDI technique can be optimized for a specific analyte.

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