JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2005; **40**: 1590–1594
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jms.937

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MALDI-TOF mass spectrometry of a combinatorial peptide library: effect of matrix composition on signal suppression†

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Received 9 August 2005; Accepted 12 September 2005

The effect of matrix composition on signal suppression caused by a dominant compound under MALDI ionization was studied using the combinatorial TQTXT pentapeptide library as a model system. The peptide library is composed of 19 components with all proteinogenic amino acids except cysteine in position X. From these compounds, only the Arg peptide (TQTRT) was detected with sufficient intensity in the MALDI-TOF mass spectrum under typical MALDI conditions (CCA matrix). The analysis of a set of compounds utilized as different matrix components, additives and a cationizing agent revealed that the composition of the matrix is a critical point in signal suppression. Highly improved ion yields were achieved by using a CCA/DHB mixture as a matrix. The addition of K^+ as a cationizing agent to the CCA matrix resulted in MALDI-TOF mass spectra with relative ion intensities very similar to those obtained by electrospray ionization. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: combinatorial chemistry; MALDI; mass spectrometry; peptide library; signal suppression

INTRODUCTION

Synthesis of multicomponent peptide libraries by the split and mix method was originally developed by Furka and co-workers in 1988.[1,2] Since that time, combinatorial chemistry has become a widely used tool in drug discovery for producing and high-throughput screening of a great number of compounds.[3,4] Analytical characterization of compound mixtures derived from 'natural' (e.g. protein digests) or from 'synthetic' (e.g. combinatorial) origin raises diverse methodological problems. Owing to its selectivity, sensitivity and versatility, mass spectrometry is a widely used high-throughput analytical technique in mixture analysis, offering diverse application areas and accurate and fast identification of analytes. Electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are the most widely applied ionization techniques in biomolecular mass spectrometry, since these allow sensitive and rapid identification of intact biomolecules even in complex mixtures. However, analysis of a 'natural', biological mixture can be significantly different from the analysis of 'synthetic', chemical mixtures, which could mean not only a different contamination pattern but also the purpose of the measurements. For example, for protein identification from tryptic digests, the identification of all components is usually not necessary, while in combinatorial chemistry the main task is the unambiguous identification of all compounds present in the mixture.

In mass spectrometric analysis of combinatorial libraries, the ionization method is almost exclusively ESI.[5] This method provides similar ionization efficiency for each single compound present, which makes the theoretical prediction of the mass spectrum possible.[6] Comparison of computer-predicted and measured mass distributions could detect side products, indicating incomplete coupling or deprotection. In the case of smaller libraries with a few hundred components, complete assignment is possible by high-resolution measurements by FT-ICR mass spectrometry. Ultrahigh resolution FT-ICR mass spectrometry offers an approach for distinguishing peptide isobars having similar molecular mass.[7] HPLC-coupled mass spectrometry or tandem mass spectrometry also makes detailed analysis of peptide libraries possible. This includes, for example, the detection of side products and differentiation of isomer or isobar ions, even by using low resolution instrumentation.[8]

MALDI ionization has also been occasionally used for analysis of combinatorial peptide libraries;[3] however, it did not become widespread in the analytical practice.

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†Paper presented at the 23rd Informal Meeting on Mass Spectrometry, Fiera di Primiero, Italy, 15–19 May 2005.
Contract/grant sponsor: Hungarian Research Fund (OTKA);
Contract/grant numbers: T043538; T043576.
Contract/grant sponsor: Ministry of Education of Hungary NKFP MediChem2; Contract/grant number: 1/A/005/2004.

The main advantages of MALDI-TOF mass spectrometry are the sensitivity, the small amount of sample required and easy automation, which make the technique ideal for high-throughput applications. The improved resolution in reflectron mode and the possibility of tandem mass spectrometric measurement by postsource decay or by collision-induced dissociation (e.g. using Q-TOF or TOF-TOF analyzers) provides detailed structural assignments of each single component present in mixtures. 'On-bead' direct laser desorption/ionization of compounds attached to the support by a covalent bond is possible by using various photosensitive linkers, even from a single bead, which is particularly useful in the deconvolution of the active compounds. However, an important disadvantage of MALDI ionization is that the detected ion signals that originated from the individual species in a mixture usually do not reflect their respective concentration. In some cases, a certain component (e.g. present in high concentration) suppresses the signal of the other compounds, which makes the interpretation of the mass spectra difficult, and prevents the identification of all components in the mixture.

A crucial problem of MALDI ionization is that fundamentals of ion generation and the desorption process are poorly understood, and signal suppression is commonly observed in mixture analysis. The signal suppression effect refers to the difference in MALDI ionization efficiency. Incorporation of charged residues or the presence of basic amino acids may increase the relative MALDI ion yield. However, charge competition, and therefore the basicity–acidity, is not the only determinant in defining the relative desorption/ionization efficiencies. Relative intensities are also influenced by the conformation and hydrophobicity of the analyte (determining its incorporation into matrix crystals), as well as by matrix properties and the gas-phase proton affinity of the matrix and the analyte. The probability of signal suppression effects increases with increased analyte complexity, and optimal sample preparation becomes important especially in the analysis of mixtures such as chemical compound libraries and protein digests.

The success of a MALDI measurement highly depends on the type of the matrix and also on the sample preparation. Selection of these experimental conditions is still an empirical procedure. The aim of this work was to compare various compounds as matrix materials for MALDI analysis of a combinatorial peptide library. Using the same peptide library, we compared the relative ionization efficiencies of peptides in spectra obtained by MALDI or ESI. In these experiments, the effect of matrix on signal suppression was studied using a set of different matrixes, additives and a cationizing agent.

The pentapeptide library used in this study was synthesized by the portioning–mixing approach. Sequences are based on the TQTXT epitope motif of the mucin-2 gastrointestinal glycoprotein (MUC2).[9,10] This well-characterized peptide library is composed of 19 components, with all the proteinogenic amino acids in position X. For synthetic reasons, cysteine was excluded. This library was characterized before on the basis of the peptide content, amino acid composition, ESI mass spectrometry and HPLC-MS, indicating that all the 19 components are present in the library in similar quantities.[7,8]

EXPERIMENTAL

Acetonitrile was purchased from Merck and reagents and matrices were purchased from Sigma-Aldrich. In the experiments, MilliQ-purified water was used (gradient grade).

The TQTXT peptide library was synthesized by the portioning-mixing method on solid phase using Boc/Bzl methodology, as described recently,[7] and analyzed without further purification. Positive ESI mass spectrum was acquired on a Q-Tof Micro (Micromass, Manchester, UK) hybrid, quadrupole-orthogonal acceleration time-of-flight instrument in positive ionization mode. The following instrumental settings were used: capillary voltage 3000 V, sample cone voltage 30 V, desolvation temperature 180 °C and ion source temperature 80 °C. The sample was dissolved in acetonitrile : water = 1 : 1 (v/v) mixture, containing 0.1% acetic acid at a concentration of 0.5 mg/ml, and was analyzed by direct sample infusion at a flow rate of 5 μl/min.

MALDI mass spectra were acquired on a Voyager DE-Pro MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA), equipped with a $\lambda = 337$ nm nitrogen laser in positive, reflectron acceleration mode. The TQTXT peptide library was dissolved in acetonitrile : water = 1 : 1 (v/v) mixture at a concentration of 0.5 mg/ml. 0.5 μl sample solution was mixed with 0.5 μl matrix solution, placed onto the target and allowed to dry on air. Mass to charge (m/z) calibration was performed externally using a set of peptides as standard (Perseptive Biosystems). Spectra were acquired by 20 kV acceleration voltage, 75% grid voltage, 0.005% guide wire and 120 ns delay time in the range of m/z 150–1500.

Matrices used in this study were α-cyano-4-hydroxycinnamic acid (CCA) and 2,5-dihydroxybenzoic (DHB) acid, which were dissolved in acetonitrile : water = 1 : 1 (v/v), 0.1% trifluoroacetic acid (v/v) mixture at a concentration of 10 mg/ml. 2′,4′,6′-trihydroxyacetophenone (THAP) (3 mg/ml) and 6-aza-2-thiothimin (ATT) (5 mg/ml) matrices were dissolved in acetonitrile : water = 1 : 3 (v/v) mixture, containing 25 mM ammonium citrate. CCA/DHB mixture was prepared by 1 : 1 mixing of the matrix solutions. In the case of CCA matrix, the following additives were tested: 20 mM ammonium citrate and 0.2, 1 and 2 mM KCl.

In the case of CCA or CCA/DHB matrix, spectra were obtained by randomly scanning the sample surface. In other cases, samples containing crystals were searched on the target surface ('sweet' spots). Approximately 300 spectra were averaged to improve signal-to-noise ratio.

RESULTS

A pentapeptide library based on the TQTXT epitope motif of MUC2 glycoprotein, containing 19 components with all proteinogenic amino acids except cysteine in position X, was analyzed by MALDI-TOF mass spectrometry, and the effect of matrix composition on signal suppression was studied.

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Ionization efficiencies were studied and compared for electrospray and for MALDI ionization. In the ESI mass spectrum (Fig. 1(A)), the peptides were detected with highly similar relative intensities. The isomer peptides TQTIT and TQTLT appeared as a single peak with double intensity. The peak overlapping that was present at this resolution in the case of isobar or ^{13}C isobar peptides also influenced the relative intensities. Variations in ion intensities could also be explained by a slightly different concentration of the individual peptides due to synthetic reasons. However, the ESI mass spectrum indicated similar relative ion yields for all peptides. In addition, most of the library components can be easily identified from a single mass spectrum. In contrast, spectra from MALDI ionization experiments (Figs 1(B) and (C)) show a characteristic suppression effect on relative ion yields, which was investigated in detail using a set of matrix compounds.

Most frequently, CCA matrix is used for MALDI ionization of peptides or low molecular weight proteins. However, in the presence of CCA matrix, we were able to detect only peptide TQTRT with sufficient intensity in the MALDI-TOF mass spectrum of the TQTXT peptide library (Fig. 1(B)). This peptide corresponds to the most basic component of the mixture. Beside this, only a few other peaks appeared in the mass spectrum. Also, these ions were present as sodium adducts, and they exhibited a relative intensity of less than 10% compared to the Arg peptide. On the basis of this MALDI mass spectrum, none of the library components (except TQTRT) can be identified unambiguously.

Ammonium salts, like ammonium citrate, added to the CCA matrix at low concentration may increase the intensity of tryptic peptides and can enhance the signal-to-noise ratio.[11] By adding 20 mM ammonium citrate salt to the CCA matrix, the MALDI spectrum of the TQTXT peptide library changed significantly (Fig. 1(C)). First, the absolute intensity of the TQTRT peptide increased to a great extent (approximately 20-fold), and this ion produced an excellent signal-to-noise ratio (~3000). On the other hand, none of the other TQTXT peptides was detected, since all the other peaks disappeared completely. This result clearly shows the importance of the optimization of experimental conditions in mixture analysis by MALDI and might indicate the differences of 'natural' and 'synthetic' peptide mixtures.

The suppression effect in the TQTXT peptide library was further studied in the presence of various matrices. Beside CCA, DHB matrix is used commonly as a MALDI matrix in peptide analysis and in proteomics. With DHB matrix, an improvement in ion yields was observed for most of the peptides in the TQTXT library (Fig. 2(A)). The two most intensive ions in the MALDI-TOF spectrum corresponded to the protonated molecular ions of peptides TQTRT and TQTWT. In addition, further components of the library were identified as protonated or sodiated molecular ions, but only with fairly low intensities. Next we performed an analysis with THAP matrix, which is also used for, e.g. the analysis of oligonucleotides.[12] Interestingly, we found that most of the components of the peptide library were detectable in the MALDI-TOF mass spectrum as protonated molecular ions (Fig. 2(B)). Although these ions exhibited quite low

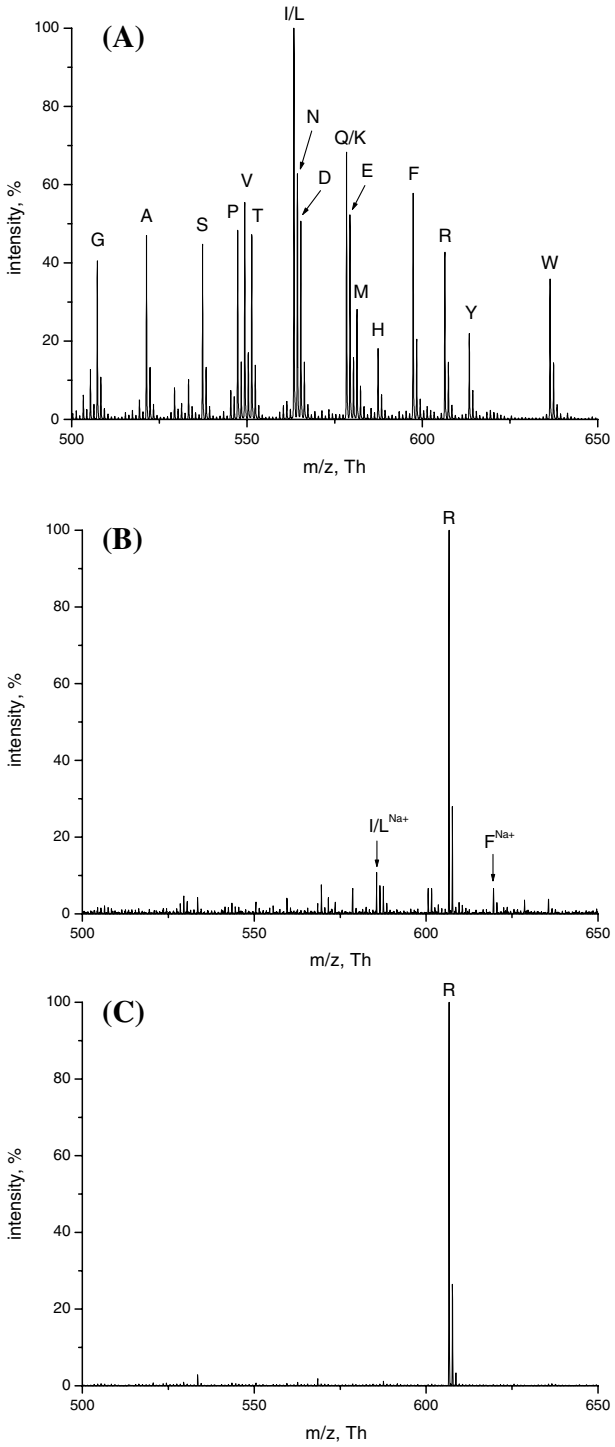

Figure 1. Mass spectra of the TQTXT peptide library acquired after (A) electrospray ionization, (B) MALDI ionization in the presence of CCA matrix and (C) MALDI ionization in the presence of CCA matrix and 20 mM ammonium citrate. The alphabets in the figures represent amino acids in position X; formation of sodium adducts are indicated by superscript letters.

relative intensities (10–20%) compared to the peak of peptide TQTRT, the signal-to-noise ratio was improved. It should be noted that the peptides could be detected despite the strong signal suppression. In MALDI mass spectrometry, ATT is rarely used; this nonacidic matrix compound is applied

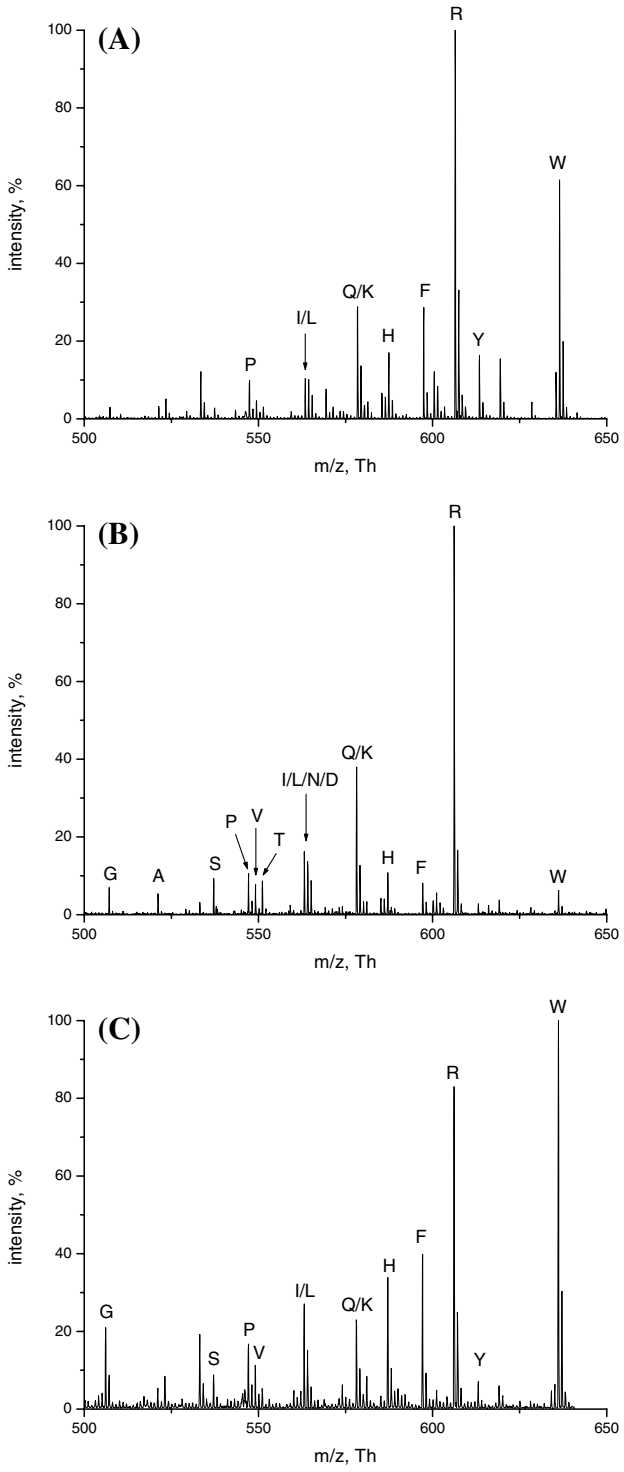

Figure 2. MALDI-TOF mass spectra of the TQTXT peptide library using (A) DHB, (B) THAP or (C) ATT as matrix compounds. The alphabets in the figures represent amino acids in position X; formation of sodium adducts are indicated by superscript letters. ^{13}C isobar peptides are not marked individually.

mainly for the detection of noncovalent complexes.[13,14] In the presence of ATT matrix, the MALDI-TOF spectrum obtained with the peptide library showed a completely different pattern. In contrast to the measurements described above, the most intensive peak in this spectrum corresponds to the peptide containing tryptophane in position X (TQTWT), and the highly basic Arg peptide (TQTRT) was only the second in intensity (Fig. 2(C)). It is important to note that peptide TQTWT was also a characteristic intensive peak in the MALDI spectrum in the presence of DHB matrix. This phenomenon might be explained by the higher UV absorption of Trp-peptide compared to others at the wavelength of the laser ($\lambda = 337$ nm).

Mixture of CCA and DHB matrices for peptide and protein analysis is a relatively new and promising combination published by Laugesen and Roepstorff in 2003.[15] A marked increase in relative intensities and equalization between the ion intensities in the case of tryptic peptides possessing arginine and lysine at terminal position(s) were reported. Application of a 1 : 1 mixture of CCA and DHB matrixes for the analysis of the TQTXT peptide library resulted in an improved spectrum as compared to the spectra corresponding to the respective individual matrices (Fig. 3). In this case, all components of the peptide library were detected as sodiated molecular ions having quite similar relative intensities, apart from peptide TQTRT, of which protonated ion is still the base peak in the spectrum.

Next we have studied the effect of the cationizing agent on the signal suppression phenomenon in the MALDI-TOF spectrum of the TQTXT library, which was investigated by adding KCl salt to CCA matrix at low concentrations. In the presence of 0.2 mM K^+ ion, we observed the formation of intensive potassiated molecular ions derived from all components. Increasing the K^+ concentration resulted in elevated relative intensities of the peptides. In the presence of 1 mM K^+, the potassiated molecular ions of most of the peptides reached the 30–40% relative intensity, compared to the protonated molecular ion of the Arg peptide (Fig. 4(A)). Further increase of the K^+ concentration to 2 mM resulted in the significant suppression of the signal corresponding to the protonated TQTRT peptide (Fig. 4(B)). In this case, the relative intensities in the MALDI spectrum become remarkably similar to those obtained in the ESI mass

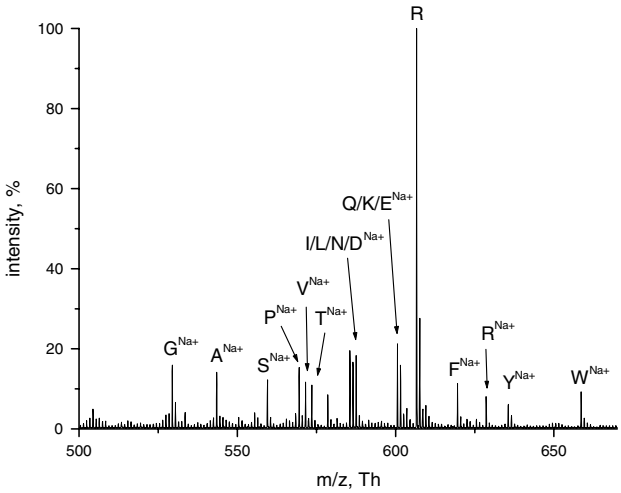

Figure 3. MALDI-TOF mass spectrum of the TQTXT peptide library in the presence of CCA/DHB matrix mixture. The alphabets in the figure represent amino acids in position X; formation of sodium adducts are indicated by superscript letters. ^{13}C isobar peptides are not marked individually.

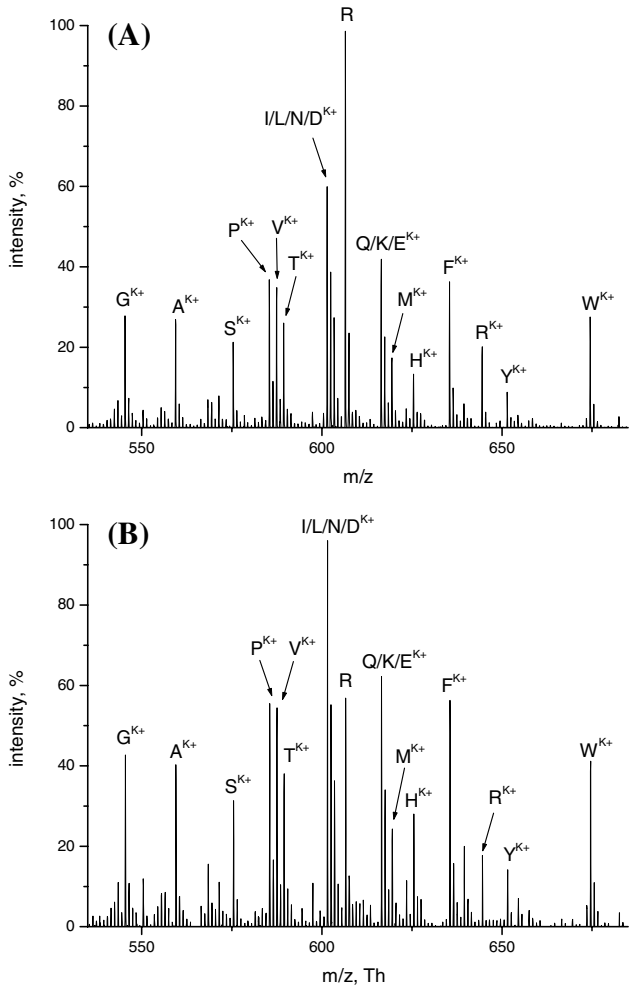

Figure 4. MALDI-TOF mass spectrum of the TQTXT peptide library in the presence of CCA matrix and KCl in a concentration of (A) 1 mM and (B) 2 mM. The alphabets in the figures represent amino acids in position X; formation of potassium adducts are indicated by superscript letters. ^{13}C isobar peptides are not marked individually.

spectrum. Our result shows that the signal suppression effect caused by a dominant compound in a mixture could be reduced significantly by adding a cationizing agent like K^+ to the matrix.

CONCLUSION

The suppression effect in MALDI ionization was studied using the combinatorial TQTXT pentapeptide library as a model system. The peptide library is composed of 19 components from which only peptide TQTRT was detected with sufficient intensity in the MALDI-TOF mass spectrum in the presence of CCA matrix. The analysis of a set of matrix compounds revealed that the composition of the matrix is a critical point in signal suppression. We observed the lowest ion yields compared to the Arg peptide in the case of CCA matrix. These values were even further decreased by the addition of ammonium salt. In the presence of ammonium citrate, none of the library components except peptide TQTRT was detected. Significantly improved ion yields were achieved by using other matrix compounds. The best results were obtained by using CCA/DHB mixture; however, the application of DHB, THAP or ATT matrices alone also provided some advantages. Interestingly, the relative intensity of the TQTWT peptide was markedly increased in DHB and ATT matrices. Generation of potassiated molecular ions by adding potassium salt to the matrix leads to an improved detection of the library components to a significant extent. Taken together, we conclude that by appropriate combination of matrix compounds and salt additives, the relative intensities observed in MALDI spectrum became similar to those measured in ESI.

Acknowledgements

This work was supported by the grants of the Hungarian Research Fund (OTKA) T043538 and T043576 and by the Ministry of Education of Hungary NKFP MediChem2 1/A/005/2004.

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