

Adduct simplification in the analysis of cyanobacterial toxins by matrix-assisted laser desorption/ionization mass spectrometry

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A novel method for simplifying adduct patterns to improve the detection and identification of peptide toxins using matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry is presented. Addition of 200 μ M zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) to samples prior to spotting on the target enhances detection of the protonated molecule while suppressing competing adducts. This produces a highly simplified spectrum with the potential to enhance quantitative analysis, particularly for complex samples. The resulting improvement in total signal strength and reduction in the coefficient of variation (from 31.1% to 5.2% for microcystin-LR) further enhance the potential for sensitive and accurate quantitation. Other potential additives tested, including 18-crown-6 ether, alkali metal salts (lithium chloride, sodium chloride, potassium chloride), and other transition metal salts (silver chloride, silver nitrate, copper(II) nitrate, copper(II) sulfate, zinc acetate), were unable to achieve comparable results. Application of this technique to the analysis of several microcystins, potent peptide hepatotoxins from cyanobacteria, is illustrated. Copyright © 2007 John Wiley & Sons, Ltd.

Cyanobacteria (blue-green algae) occur naturally in all kinds of waters. Many species, but not all, can produce toxins that are harmful to humans and wildlife through damage to the nervous system or liver. When blooms of these organisms generate potentially dangerous levels of toxin in the water, public notification and restriction of access must be achieved as rapidly as possible. Analysis of water samples for these toxins is a key component in the public health management of harmful algal blooms.¹

The most common cyanotoxins in freshwater systems are the microcystins (MCs), produced by several cyanobacterial genera including *Microcystis*, *Anabaena*, and *Planktothrix*. Microcystins are cyclic peptides containing seven amino acids, including five that are unusual in natural peptides (Fig. 1). More than 70 MC congeners have been identified to date.² Amino acid variations are known at every position; the most common are L-amino acid substitutions at positions 2 and 4, as well as demethylation at positions 3 and 7. Substitutions at positions 2 and 4 give rise to the naming system; e.g., a congener with leucine (L) at position 2 and arginine (R) at position 4 is known as microcystin-LR. The unique amino acid Adda in position 5, known to be required for toxicity, fits into the hydrophobic cavity of the target

protein phosphatases.^{3,4} The congeners vary from nontoxic (e.g. [(6Z)-Adda⁵] MC-LR, LD₅₀ >1200 μ g/kg) to highly toxic (e.g. MC-LR, LD₅₀ = 50 μ g/kg),^{5,6} and exhibit molecular weights (MWs) ranging from 900 to 1100 Da.

Several analytical techniques are currently used for detection and quantitation of MCs, including high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assays (ELISA), and the protein phosphatase inhibition assay (PPIA). These are currently the best available techniques for the analysis of MCs and are extensively used in the authors' water quality testing program. However, these techniques have several limitations, including extensive sample handling and lengthy analytical protocols. Quantitation by means of ultraviolet detection is inherently problematic due to the differing absorption coefficients of MC congeners⁷ and the fact that very few standards are available. HPLC, LC/MS, and ELISA provide limited information on potential toxicity of the sample since congeners often generate responses not correlated with their toxicity.^{8–11} Additionally, when HPLC, ELISA, and PPIA have been used to analyze identical samples, the results sometimes show poor agreement between the methods, particularly when congeners other than MC-LR are present.^{12–14} Development of alternative analytical methods could improve prediction of and response to harmful algal blooms.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has the potential to overcome many

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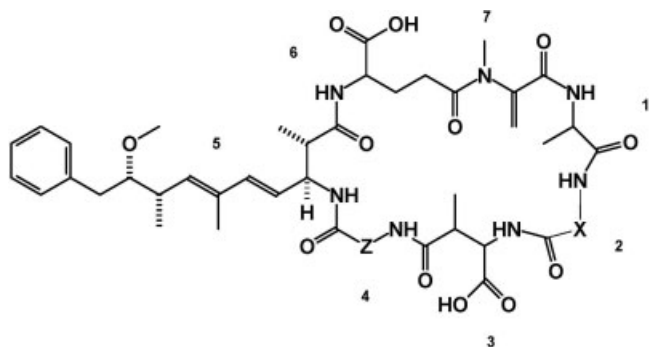


Figure 1. The generic structure for microcystin congeners is *cyclo*-(D-alanine¹-X²-D-MeAsp³-Z⁴-Adda⁵-D-glutamate⁶-Mdha⁷) in which X and Z are variable L-amino acids, D-MeAsp³ is D-erythro-β-methylaspartic acid, Adda⁵ is (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and Mdha⁷ is N-methyldehydroalanine.

of the disadvantages presented by the current methods. It is an extremely rapid, high-resolution, sensitive technique that requires little sample handling, is tolerant of contaminants, and allows identification of toxin congeners. These advantages have led a number of researchers^{14–17} to utilize MALDI in the detection of MCs. In each case, however, MALDI was used only for qualitative detection and identification of toxin congeners. PPIA, ELISA, and/or HPLC were utilized when quantitation was desired. To our knowledge, quantitation of cyanobacterial toxins using MALDI-MS has never been reported.

Unfortunately, quantitation issues have plagued MALDI since its early days. As with electrospray ionization (ESI)-MS, one area of concern is the potential for several different ionization pathways, including protonation and cationization (formation of sodium and/or potassium adducts), to occur during analysis.¹⁸ The dominant peak cluster generated during MALDI analysis is dependent on analyte structure. Peptides containing basic amino acids such as arginine (e.g. MC-LR) appear primarily as protonated molecules,^{19,20} while the absence of convenient protonation sites, as with cyclosporin A (CsA)²¹ or nonpolar MCs,²² enhances cationization. The presence of acidic groups can also enhance cationization;²³ this is seen with MCs, particularly those lacking arginine or other basic residues such as MC-LF (cf. Figs. 2 and 3). Multiple adduct peaks (e.g. [M+H]⁺, [M+Na]⁺, and [M+K]⁺) often occur for a single analyte, dividing and thus weakening the overall signal, hindering detection, and complicating attempts at quantitation. Complex spectra can be a significant problem for MCs, with more than 70 variants clustered in the MW range from 900 to 1100 Da and most field samples containing several congeners, each of which may exhibit several adduct peaks. Mass overlap occurs in several instances between a sodium adduct from one MC and the protonated molecule of another, which could significantly hinder congener identification using MALDI-time-of-flight (TOF)MS.

Simplification of these adduct patterns through removal of the sodium and potassium adducts should enhance detection and identification of congeners and might also be an effective first step in achieving quantitation of MCs by MALDI-

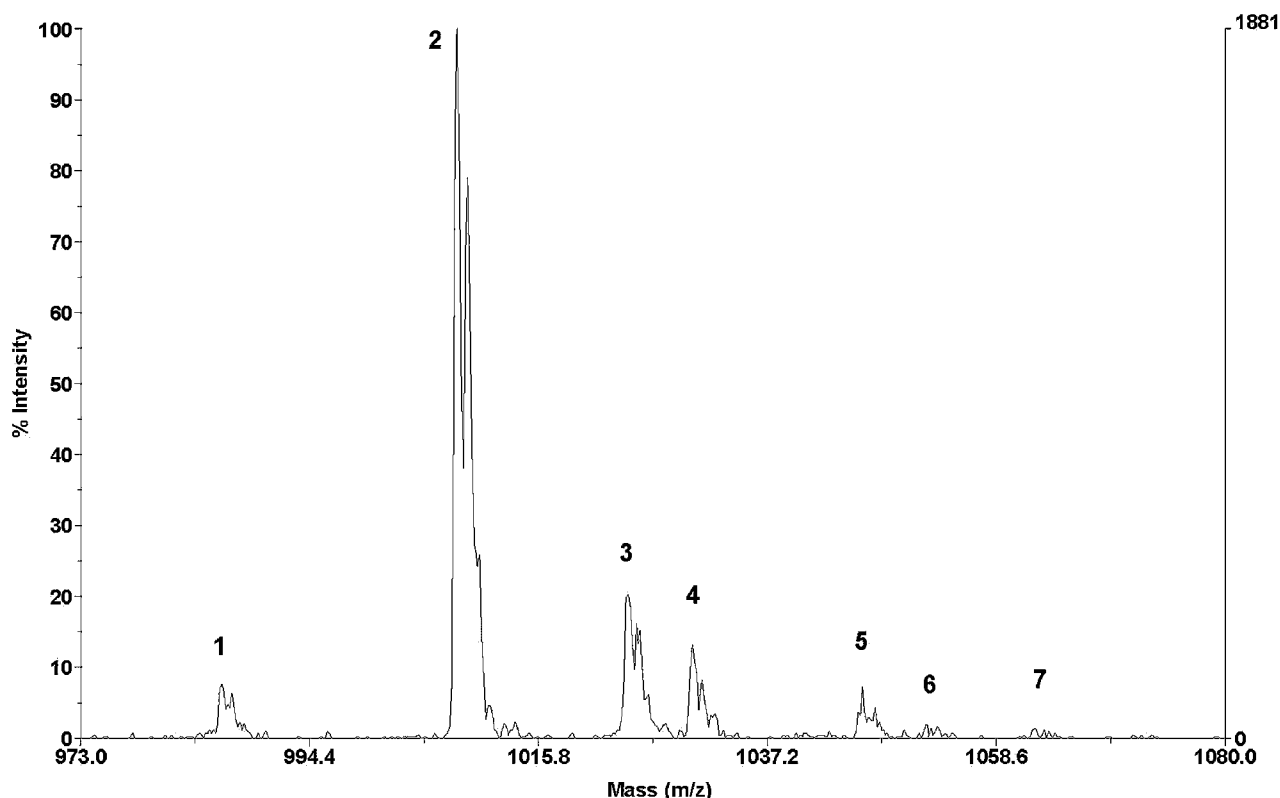


Figure 2. The mass spectrum of 100-μM MC-LF standard solution. 1: [MCLF+H]⁺; 2: [MCLF+Na]⁺; 3: [MCLF+K]⁺; 4: [MCLF-H+2Na]⁺; 5: [MCLF-H+Na+K]⁺; 6: [MCLF-2H+3Na]⁺; 7: [MCLF-H+2K]⁺.

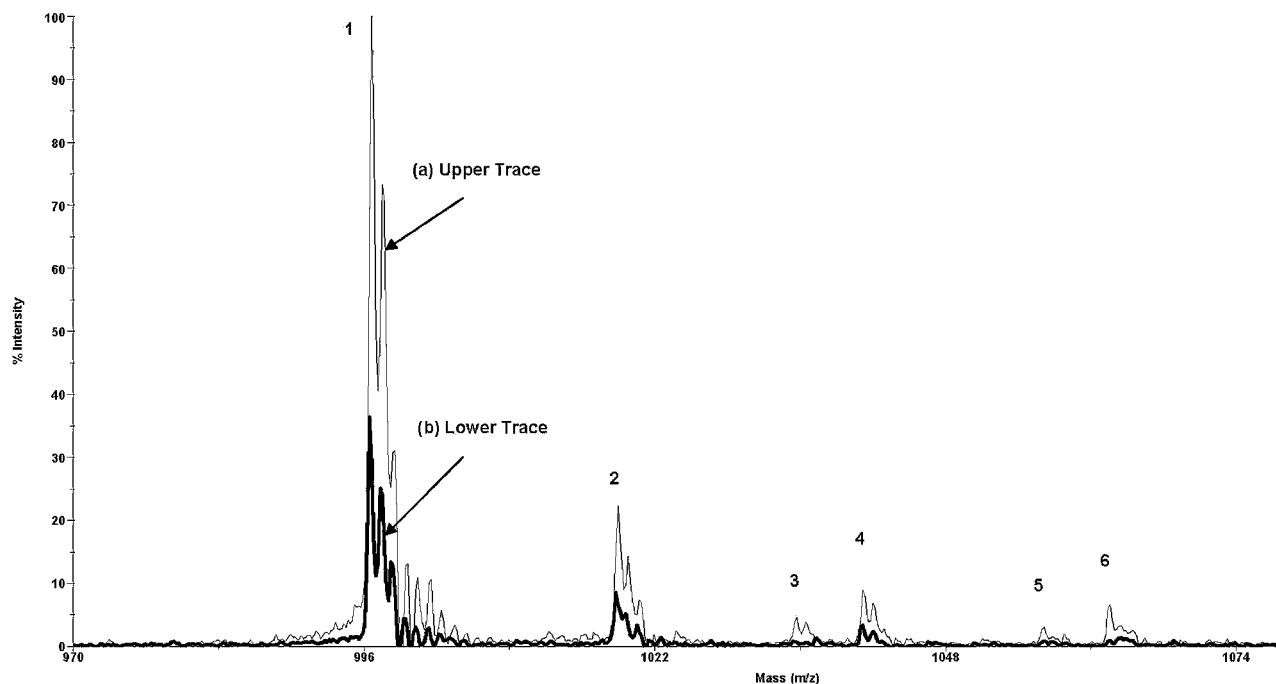


Figure 3. Positive ion MALDI mass spectra of (a) 50 μM MC-LR and (b) 50 μM MC-LR + 10 mM 18-crown-6. 1: $[\text{MCLR}+\text{H}]^+$; 2: $[\text{MCLR}+\text{Na}]^+$; 3: $[\text{MCLR}+\text{K}]^+$; 4: $[\text{MCLR}-\text{H}+2\text{Na}]^+$; 5: $[\text{MCLR}-\text{H}+\text{Na}+\text{K}]^+$; 6: $[\text{MCLR}-2\text{H}+3\text{Na}]^+$.

TOFMS. Removing cationization as a competitive pathway has been the focus of much research to improve signal strength and simplify quantitation during MALDI-MS. Desalting samples prior to analysis through the use of micro solid-phase extraction (C18 sorbent in a micropipette tip)^{24,25} or washing the sample spots after drying on the target^{24,26–30} have both been used with some success. However, additional sample handling steps counteract the simplicity inherent to MALDI analysis, and poor recovery of some peptides, particularly trace components, has been noted.^{27,29} The use of the cyclic polyether 18-crown-6 to complex sodium and potassium ions in solution has been shown to dramatically reduce cation adducts during the analysis of trypsin digests³¹ and intact bacterial cells.^{32,33} Crown ethers have also been incorporated into a sol-gel material to generate a desalting substrate for MALDI sample deposition.³⁴ Other conditions tested for suppression of cation adducts include addition of surfactants^{26,28} and ammonium salts^{29,35,36} to the matrix, and the use of ionic liquids in place of solid matrices.^{37,38}

Some researchers have taken the opposite approach and attempted to force the formation of particular cation adducts through the addition of metal salts. This is particularly common in the MALDI analysis of neutral compounds such as polymers,³⁹ which are otherwise not efficiently ionized. A related approach⁴⁰ added lithium chloride to the matrix for the analysis of oligosaccharides, resulting in the suppression of proton, sodium and potassium adducts and enhancement of a single $[\text{M}+\text{Li}]^+$ signal.

This study assesses some of these techniques for the analysis of MCs, and presents a simple, highly effective method that simplifies congener identification by removing cation adducts and enhancing detection of protonated molecules.

EXPERIMENTAL

Materials

Microcystin-LR (MC-LR), microcystin-LF (MC-LF), and cyclosporin A (CsA) were obtained from Alexis (San Diego, CA, USA); microcystin-RR (MC-RR) and microcystin-YR (MC-YR) from CalBioChem/EMD Biosciences (La Jolla, CA, USA); ethanol from Pharmco (Brookfield, CT, USA); methanol and acetonitrile from Mallinckrodt Baker (Phillipsburg, NJ, USA); potassium chloride from EM Science (Gibbstown, NJ, USA); lithium chloride, copper(II) nitrate trihydrate, and copper(II) sulfate pentahydrate from Fisher Scientific (Fair Lawn, NJ, USA); and 18-crown-6 ether, α -cyano-4-hydroxycinnamic acid (CHCA), silver nitrate, sodium chloride, trifluoroacetic acid (TFA), zinc acetate dihydrate, and zinc sulfate heptahydrate from Sigma-Aldrich (St. Louis, MO, USA). All materials were used as received.

Mass spectrometry

Analyses were conducted using a Voyager-DE linear MALDI-TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a 337-nm pulsed nitrogen laser. Spectra were obtained in positive ion mode with accelerating voltage 19 kV, low mass gate setting m/z 400, delay time 75 ns, 60 laser shots per spectrum, and digitizer vertical scale set at 1000 mV. Replicate samples were spotted as 1.5- μL aliquots on a 100-well, stainless steel MALDI target and allowed to air dry; data are reported as averages of the replicate samples. Peak heights and peak areas were both examined, with similar results; for convenience, only peak height data are presented.

Sample preparation

Best results were obtained when samples were prepared in 50:50 (v/v) methanol/0.1% TFA; unless otherwise indicated, each sample contained 50 μ M MC-LR and 50 μ M CsA. Additives were included with limited volume change by combining 50 μ L of MC-LR/CsA sample with 1 μ L of 18-crown-6 ether (1, 10, or 100 mM) in 50% acetonitrile/0.1% TFA, or with 1 μ L of various metal salts (0.2–6 mM) in deionized water. Control solutions were prepared by adding 1 μ L of the corresponding solvent with the additive omitted. Resulting samples were then combined 1:1 (v/v) with CHCA (15 mg/mL in 60% ethanol/36% acetonitrile/4% deionized water) and bath sonicated (50 W) for 3 min prior to spotting on the target. Sample/matrix mixtures had a pH of approximately 3 when measured without salt or other additives.

RESULTS AND DISCUSSION

Chelation

The polyether 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane, C₁₂H₂₄O₆) is one of a group of macrocyclic compounds that can complex alkali metal ions in the central cavity. MC-LR under normal conditions generated the [MCLR+H]⁺ signal (*m/z* 995) as the main peak in MALDI analysis, with additional small alkali metal adduct peaks (Fig. 3), while CsA typically appeared as [CsA+Na]⁺ (*m/z* 1225) with small protonated and potassium adduct peaks (Fig. 4). It is evident from the spectra and numeric data (Table 1) that no significant reduction in cation adducts resulted from the addition of 18-crown-6, even though significant molar excesses were used. It is noteworthy that the average signal strengths were highest and the coefficients of variation (CVs) were lowest in the absence of 18-crown-6.

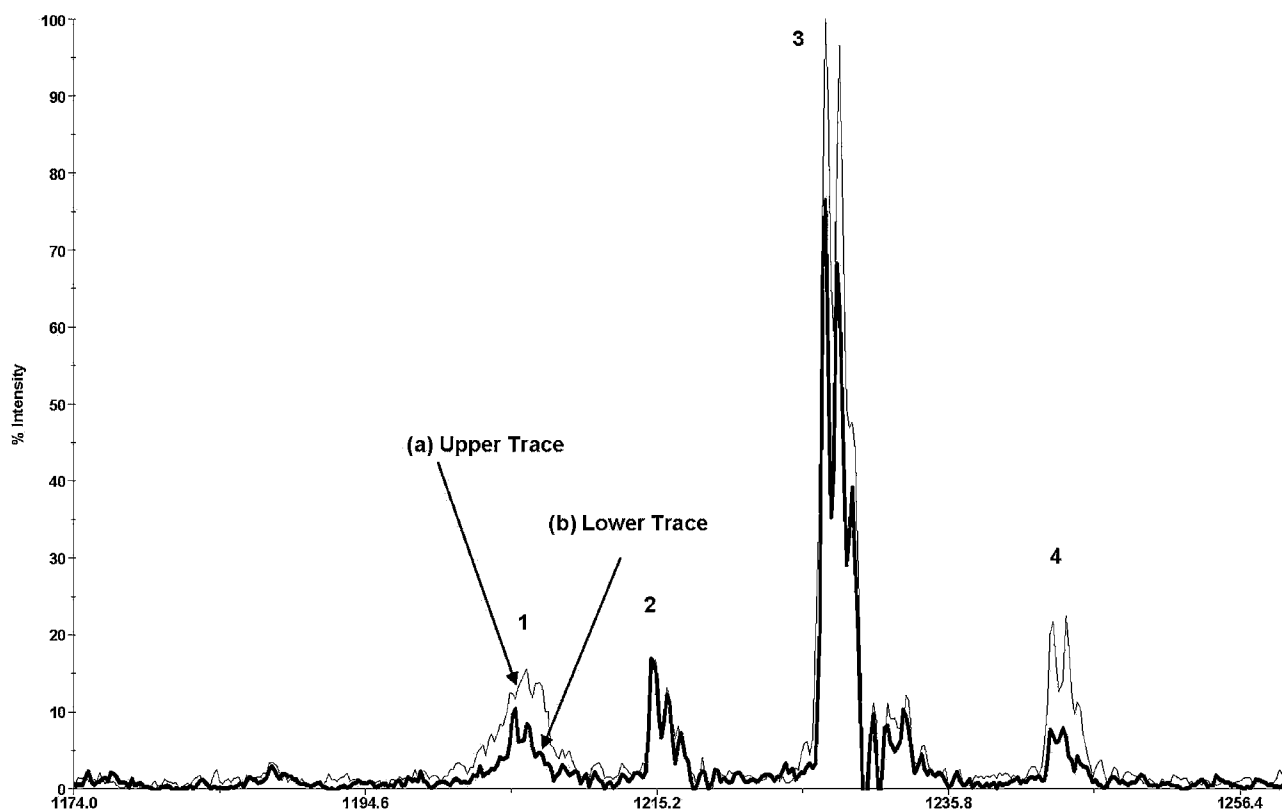


Figure 4. Positive ion MALDI mass spectra of (a) 50 μ M cyclosporin A and (b) 50 μ M cyclosporin A + 10 mM 18-crown-6. 1: [CsA+H]⁺; 2: unidentified; 3: [CsA+Na]⁺; 4: [CsA+K]⁺.

Table 1. Chelation data using 10 mM 18-crown-6 ether

Addition	No. of MCLR adducts observed	Normalized [MCLR+H] ⁺ peak height	CV ^a (%)	[MCLR+H] ⁺ as % of MCLR _{total}	Normalized [CsA+H] ⁺ peak height	CV (%)
None	6	1.00	12.7	72.8	1.0	18.9
18-Crown-6	3	0.417	30.4	74.5	0.61	31.5

^a Coefficients of variation (CV) were calculated as [standard deviation/average] for triplicate samples, expressed as a percentage. Data are averages of triplicate samples. Microcystin-LR (MC-LR) and cyclosporin A (CsA) were both at 50 μ M. Peak heights were normalized by comparison to the sample with no addition of ether.

Table 2. Quantitation parameters upon addition of alkali metals. Data are averages of ten replicate samples

Addition	No. of MCLR adducts observed	Normalized [MCLR+H] ⁺ peak height	Normalized [CsA+M] ⁺ peak height
None	6	1.00	1.00 [M = Na]
LiCl	3	0.561	1.60 [M = Li]
NaCl	4	1.20	1.54 [M = Na]
KCl	4	1.62	1.99 [M = K]

Alkali metal concentrations were 2 mM. Peak heights were normalized by comparison to the sample with no added alkali metal salt.

It is likely that complexation of alkali metal cations by 18-crown-6 ether occurs in the solution phase. Therefore, the chelation test described above was repeated with an overnight incubation period after combining the samples and the crown ether, to allow for more complete complexation of alkali metal ions by the crown ether. The results were virtually unchanged except for a further deterioration in the CVs (data not shown).

Alkali metals

Intrigued by the possibility of driving the adduct pattern toward formation of one particular adduct,⁴⁰ we added lithium chloride to samples to produce concentrations of 2, 4, and 6 mM. The effect on MC-LR was limited (Tables 2 and 3); a small [MCLR+Li]⁺ signal appeared, and the [MCLR+H]⁺ signal decreased. However, CsA shifted from a dominant [CsA+Na]⁺ signal to the [CsA+Li]⁺ signal, with some improvement in signal strength. This result was expected, given the significant molar excess of Li⁺ ions and the ease with which CsA coordinates with alkali metals. CsA, a cyclic peptide with a MW of 1201.8 g/mol, was being tested as a potential internal standard compound for future quantitation. However, this compound is of importance in clinical chemistry and the results from this work may be of interest in that area as well.

Since lithium chloride addition produced limited adduct pattern simplification, sodium and potassium were tested to see if the desired effects could be achieved with a different alkali metal. In each case, CsA was driven to form a dominant adduct with the added metal (Table 3) while [MCLR+H]⁺ remained the dominant MC-LR adduct. Signal strength improved in all cases (Table 2), but the adduct patterns did not simplify as desired.

Transition metals

Amino and carboxyl groups, along with electron pairs on nitrogen and oxygen, provide potential metal coordination sites within the cyclic framework of MCs. MCs are reported^{41,42} to act as intermediate strength ligands for transition metal ions in solution, particularly Cu²⁺ and Zn²⁺, but also potentially including Ag¹⁺. The polarities of the L-amino acids at positions 2 and 4 do not have a significant effect on the binding capacity,⁴¹ implying that multiple sites on the peptide are involved in metal ion binding. This has been confirmed by Yan and coworkers,⁴² who found that the most stable aqueous complexes result from localization of the metal ion in the cavity region of the peptide.

Transition metal salts (200 μM) were added to a mixture of MC-LR and CsA (50 μM each); the results are summarized in Tables 3 and 4. Silver nitrate and silver chloride were added to mixtures containing 50 μM CsA and 50 μM MC-LR to produce final metal salt concentrations of 200 μM, 2 mM, 20 mM, 40 mM, and 60 mM. Silver chloride did not produce a silver adduct with either MC-LR or CsA. This may be due to the low solubility of AgCl, which limited the number of Ag¹⁺ ions available in solution. However, strong silver adduct signals were observed at *m/z* 1101/1103 ([MCLR+Ag]⁺) and *m/z* 1309/1311 ([CsA+Ag]⁺) upon addition of AgNO₃. The double peaks are explained by the isotopic composition of silver as a nearly even mixture of ¹⁰⁷Ag and ¹⁰⁹Ag. With respect to MC-LR, AgNO₃ addition resulted in the suppression of all other adducts except [MCLR+H]⁺, including the common fragment signal at *m/z* 861. The overall loss of signal strength for the MC-LR peaks as a group may have rendered the *m/z* 861 peak undetectable. Alternatively, this result may indicate stabilization of the Adda side chain upon addition of AgNO₃. Addition of AgCl provided no advantages in the detection of MCs; the incomplete adduct simplification

Table 3. Signals generated upon addition of metal salts to a mixture of 50 μM MC-LR and 50 μM CsA

Addition	MCLR peaks observed (<i>m/z</i> ratio)	CsA peaks observed (<i>m/z</i> ratio)
None	861, 995 , 1017, 1033, 1039, 1055, 1061	1203, 1215 ^a , 1225 , 1241
LiCl	861, 995 , 1001, 1017	1203, 1209 , 1225
NaCl	861, 995 , 1017, 1039, 1061	1203, 1225 , 1241, 1247
KCl	861, 995 , 1033, 1071, 1109	1203, 1225, 1241
AgCl	861, 995 , 1017, 1033, 1039, 1055, 1061	1203, 1215, 1225 , 1241
Ag(NO ₃) ₂	995 , 1101/1103	1203, 1215, 1225, 1309/1311
Cu(NO ₃) ₂	861, 995 , 1017, 1033, 1057	1203 , 1215, 1225, 1265
CuSO ₄	861, 995 , 1017	1203 , 1215
Zn(C ₂ H ₃ O ₂) ₂	861, 995 , 1017, 1033, 1055, 1061	1203, 1215, 1225
ZnSO ₄	861, 995	1203

^a The identity of the *m/z* 1215 peak in the CsA cluster is unknown. The final metal ion concentrations were 2 mM for the alkali metals and 200 μM for the transition metals. Peaks in **bold** were dominant within each adduct cluster.

Table 4. Quantitation parameters upon addition of transition metals. Data are averages of ten replicate samples

Addition	No. of MCLR adducts observed	Normalized [MCLR+H] ⁺ Peak Height	CV ^a (%)	[MCLR+H] ⁺ as % of MCLR _{total}	Normalized [CsA+H] ⁺ Peak Height	CV ^a (%)	[CsA+H] ⁺ as % of CsA _{total}
None	6	1.00	9.2	94.5	1.00	52.3	23.8
AgCl	6	0.815	20.8	93.2	1.66	35.2	19.0
AgNO ₃	2	0.278	27.4	85.5	2.16	39.3	36.1
Cu(NO ₃) ₂	4	1.19	11.6	95.2	1.14	37.3	98.3
CuSO ₄	2	1.15	12.5	99.9	1.18	51.1	100
Zn(C ₂ H ₃ O ₂) ₂	5	1.09	12.9	98.3	1.00	52.3	45.2
ZnSO ₄	1	1.07	8.6	100	3.66	3.60	100

^a Coefficients of variation (CV) were calculated as [standard deviation/average] for the replicate samples, expressed as a percentage. Transition metal concentrations were 200 μM. Peak heights were normalized by comparison to the sample with no added transition metal salts.

provided by AgNO₃ is offset by the significant loss of total signal strength.

The effects of copper addition were tested using 200 μM solutions of Cu(NO₃)₂·3H₂O and CuSO₄·5H₂O. Both salts produced somewhat higher average peak heights for both MC-LR and CsA. The [MCLR+H]⁺ signal was enhanced by the addition of either copper salt; copper(II) nitrate also produced a small [MCLR+Cu]⁺ adduct (Table 3). Similar results were observed for CsA. The simplified pattern produced by CuSO₄ addition may enhance quantitation, but it was not as complete as desired.

Addition of zinc acetate produced [CsA+Na]⁺ as the main CsA adduct; however, it was less dominant compared to [CsA+H]⁺ than when no metal salts were added, indicating enhancement of the protonation pathway. Zinc sulfate drives

this process to completion, producing only the [CsA+H]⁺ signal. MC-LR was also detected as a single [MCLR+H]⁺ signal when zinc sulfate was present, with the exception of a small fragment peak at *m/z* 861. Figure 5 shows the spectrum with no metal salt addition (Fig. 5(a)) and after addition of 200 μM zinc sulfate (Fig. 5(b)). Simplification of the adduct pattern down to a single peak for the protonated molecule was observed for both MC-LR and CsA. Only ZnSO₄ provided a single adduct for both MC-LR and CsA, gave improved CVs for both peak heights, and produced the strongest signal for [CsA+H]⁺. Although removal of the small MCLR fragment signal observed at *m/z* 861 would further simplify the spectrum, the [MCLR+H]⁺ peak height exhibits very low CV even when this fragmentation occurs. Additionally, the peak is in a different region of the

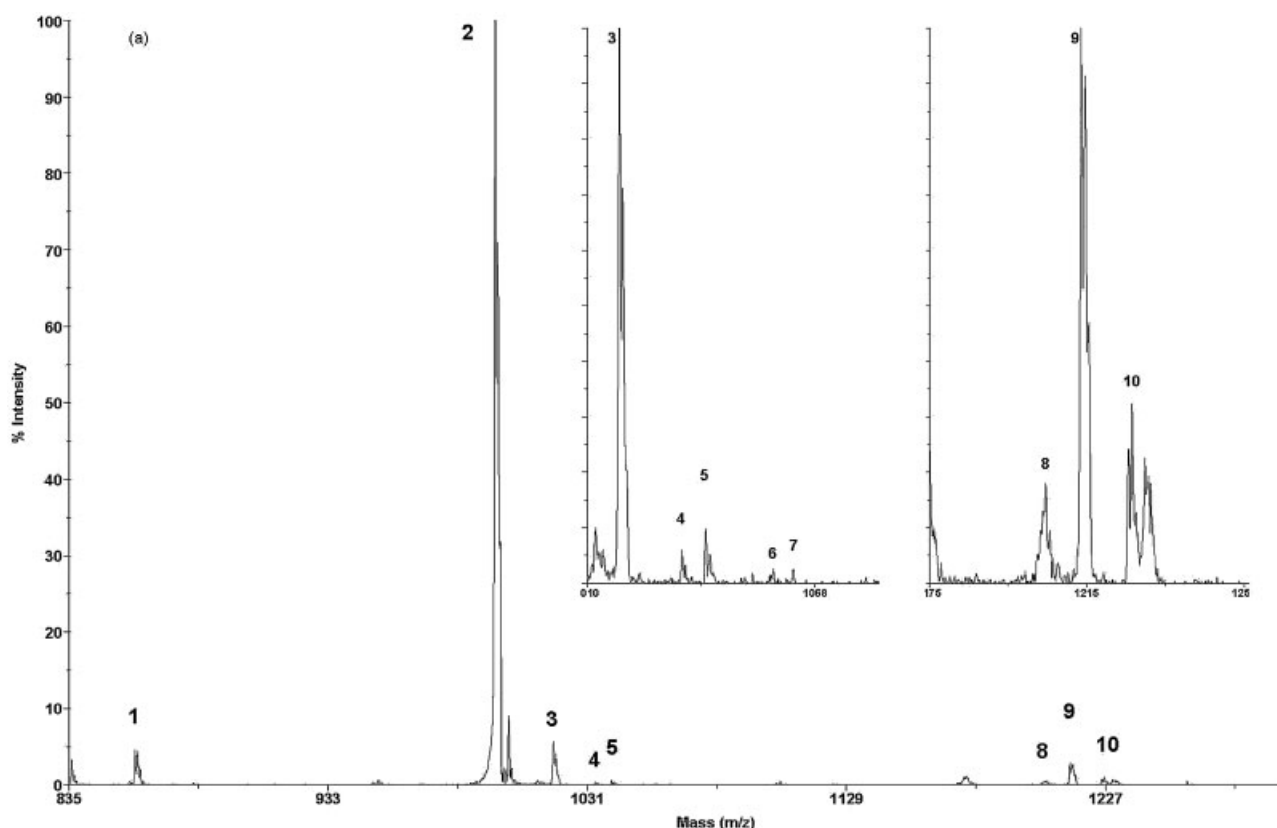


Figure 5. Positive ion MALDI mass spectra of (a) 50 μM MC-LR and 50 μM CsA, and (b) 50 μM MC-LR and 50 μM CsA plus 200 μM zinc sulfate. 1: [MCLR-134+H]⁺; 2: [MCLR+H]⁺; 3: [MCLR+Na]⁺; 4: [MCLR+K]⁺; 5: [MCLR-H+2Na]⁺; 6: [MCLR-H+Na+K]⁺; 7: [MCLR-2H+3Na]⁺; 8: [CsA+H]⁺; 9: unidentified; 10: [CsA+Na]⁺.

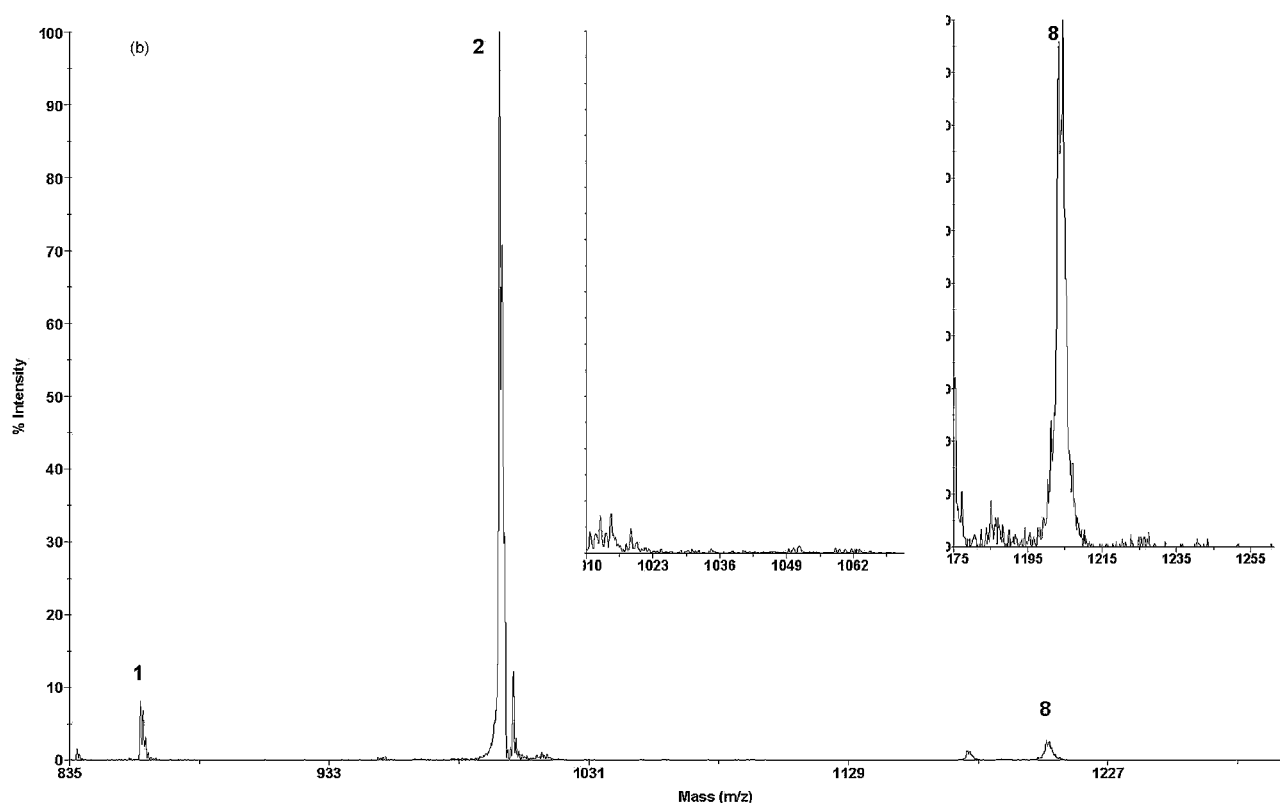


Figure 5. (Continued)

spectrum, well removed from other MC peaks. Therefore, the potential for signal overlap that may interfere with congener identification does not exist for this fragment peak, while it is a concern for sodium and potassium adducts. It is therefore possible to simplify quantitation by ignoring the m/z 861 peak without compromising the analysis.

Based upon the success achieved for MC-LR, zinc sulfate was added to solutions of several other MC congeners ranging from strongly polar (MC-RR) to nonpolar (MC-LF). The protonation pathway was enhanced for each MC upon addition of zinc sulfate (Table 5), resulting in elimination of all competing adducts for MC-YR and MC-LR, and nearly complete elimination for MC-RR. The overall signal strength (MC_{total}), peak height CV, and protonated peak percentage CV also improved dramatically for these three congeners.

For the nonpolar MC-LF, adduct simplification occurred but was incomplete, and overall signal strength (MC_{total}) decreased upon addition of zinc. CVs for peak height and protonated peak percentage showed significant improvement, in line with the results for polar MCs. The limited success of the method for MC-LF is likely due to a lack of available protonation sites and the high affinity of this congener for alkali metals. Since the majority of MCs detected in natural field samples are the polar variants,⁵ this method for enhancing the quantitation of MCs using MALDI should be widely applicable.

We hypothesize that the reduction or elimination of competing adduct signals for MCs and CsA upon zinc addition was due to the Lewis acid behavior of zinc in solution (aqueous $ZnSO_4 \cdot 7H_2O$ has a pH of 4.5). The Zn^{2+}

Table 5. Mixed microcystin congeners upon addition of zinc sulfate

MC congener	$[ZnSO_4]$ added	No. of MC adducts observed	Normalized $[MC+H]^+$ peak height	CV ^a (%)	$[MC+H]^+$ as % of MC_{total}
MC-RR	0 mM	5	1.00	47.3	90.7
	5 mM	2	1.94	23.9	99.0
MC-YR	0 mM	7	1.00	57.1	85.0
	5 mM	1	2.29	17.8	100.0
MC-LR	0 mM	7	1.00	31.1	86.3
	5 mM	1	1.31	5.2	100.0
MC-LF	0 mM	8	1.00	45.5	11.2
	5 mM	3	1.70	10.7	66.0

^a Coefficients of variation (CV) were calculated as [standard deviation/average] for the replicate samples, expressed as a percentage. Microcystin (MC) concentrations were 10 μ M; transition metal concentrations were 5 mM. Peak heights were individually normalized for each congener by comparison of the peak height with 5 mM $ZnSO_4$ to the sample with 0 mM $ZnSO_4$.

ions react with water to complex hydroxide ions, releasing free protons to solution and enhancing the protonation pathway. Further work is underway to test this hypothesis.

CONCLUSIONS

These results demonstrate that simplification of the positive-ion MALDI-TOF mass spectra for cyanobacterial toxins can be achieved through the addition of zinc sulfate. This transition metal salt suppresses formation of alkali metal adducts, producing a solitary signal for the protonated molecule. This result is observed for polar peptides (MC-LR, MC-RR, and MC-YR) as well as a nonpolar cyclic peptide with a strong predisposition toward the formation of alkali metal adducts (cyclosporin A), and to a more limited extent with a nonpolar microcystin (MC-LF). Additional benefits observed include improved run-to-run repeatability and an increase in total signal strength for improved detection of trace amounts of toxin. Chelation of trace alkali metal contaminants with 18-crown-6 ether was unable to achieve suppression of undesirable adducts. The use of other transition metal and alkali metal salts to promote the formation of single adducts also did not achieve comparable results. We have hypothesized the Lewis acid behavior of zinc in aqueous solutions as an explanation for the observed effects; experiments are underway to test this hypothesis.

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