analytical chemistry

Letter

pubs.acs.org/ac

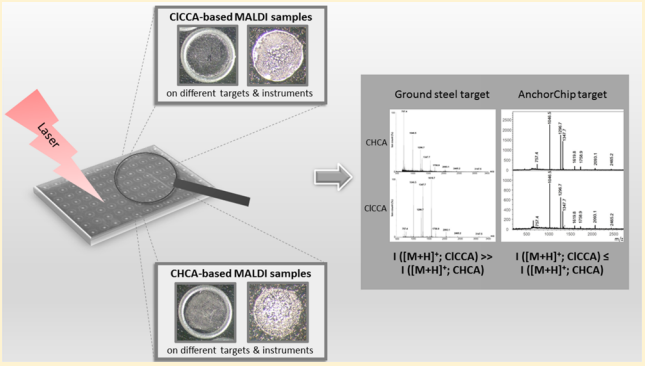
Sample Preparation: A Crucial Factor for the Analytical Performance of Rationally Designed MALDI Matrices

Kanjana Wiangnon and Rainer Cramer*

Department of Chemistry, University of Reading, Whiteknights, Reading RG6 6AD, United Kingdom

Supporting Information

ABSTRACT: Evidence is presented that the performance of the rationally designed MALDI matrix 4-chloro-α-cyanocinnamic acid (ClCCA) in comparison to its well-established predecessor α-cyano-4-hydroxycinnamic acid (CHCA) is significantly dependent on the sample preparation, such as the choice of the target plate. In this context, it becomes clear that any rational designs of MALDI matrices and their successful employment have to consider a larger set of physicochemical parameters, including sample crystallization and morphology/topology, in addition to parameters of basic (solution and/or gas-phase) chemistry.



In recent years, matrix-assisted laser desorption/ionization (MALDI)[1] has undergone some exciting developments, often based on the introduction of new matrices and/or sample preparations. New matrix systems, such as high-performing liquid MALDI matrices,[2−9] as well as the further development of commonly employed matrices for solid MALDI sample preparations[10−13] have been two areas of increased interest. The former has enabled the production of long-lasting and high-yielding analyte ion signals,[4,5,14] in some cases even the production of predominantly multiply charged MALDI ions,[4] while the latter has shown that by rational matrix design current limits of sensitivity, based on well-established matrices for solid MALDI, can be the subject for substantial further improvements.[11,12]

For analytical techniques, in particular for mass spectrometric techniques, sample preparation protocols and thus the sample's constitution can often widely vary. Consequently, this can result in a rich source of variations in analytical performance. Many parameters can influence these variations of performance, with their exact effect depending on the given conditions. In the case of MALDI MS, it is often observed that sample preparation has a major effect on the outcome of the recorded ion signal. At the sample preparation, parameters such as relative humidity, temperature, air flow, and target surface can substantially influence the MALDI sample, such as the matrix/analyte cocrystallization, and thus the resulting desorption and ion formation processes and ultimately the analyte ion signal intensities.

With this in mind, we have revisited the most promising of the above-mentioned rationally designed MALDI matrices, 4-chloro-α-cyanocinnamic acid (ClCCA),[11,12,15] and compared it with its close relative, the well-established α-cyano-4-hydroxycinnamic acid (CHCA), which has been the matrix of choice for decades in MALDI MS, mainly for the analysis of peptides.

For MALDI sample preparation, two different targets (ground stainless steel and AnchorChip) and different protocols were employed. The matrix solution preparations used were essentially the ones published by Leszyk (Leszyk preparation I and II) and the AnchorChip-specific protocol was the one recommended by Bruker in their instrument manual for the use of CHCA as matrix (Bruker preparation). For the Bruker preparation, the CHCA or ClCCA matrix solution was prepared at 1 mg/mL in 85% acetonitrile/0.1% TFA. The Leszyk preparation I is specific for CHCA, which was prepared at 5 mg/mL in 50% acetonitrile/0.1% trifluoroacetic acid (TFA), while the Leszyk preparation II is specific for ClCCA, which was prepared at 5 mg/mL in 80% acetonitrile/0.1% TFA.

MALDI sample preparation on AnchorChip targets involved making up a bulk MALDI sample solution by mixing the prepared matrix solution with the analyte solution in a ratio of 200:1 (v/v) unless otherwise reported. Aliquots of 1 μL of this bulk solution were then spotted on AnchorChip targets in triplicates. On ground steel targets, dried droplet (DD) sample preparations were prepared by spotting 0.5 μL of matrix solution first on the target and adding 0.5 μL of (diluted) analyte solution immediately afterward. In general, all MALDI samples were allowed to dry under ambient conditions for 45 min.

The analytes used for this study were a peptide standard mixture (cat. no. 222570; Bruker UK, Coventry, UK) and a bovine serum albumin tryptic digest (cat. no. PS-204-1; Protea

Received: November 26, 2014
Accepted: January 18, 2015
Published: January 19, 2015

DOI: 10.1021/ac504412p
Anal. Chem. 2015, 87, 1485−1488

Table 1. MALDI MS Analyte Ion S/N for Nine Peptides (20 fmol Each) Obtained from Samples Prepared on an AnchorChip or Ground Steel Target Plate Using CHCA or ClCCA as MALDI Matrices[a]

	average signal-to-noise ± SD[b]					
	TOF/TOF with AnchorChip target		TOF/TOF with ground steel target		Q-TOF with ground steel target	
	CHCA	ClCCA	CHCA	ClCCA	CHCA	ClCCA
bradykinin (1–7) (m/z = 757.4)	299 ± 93	132 ± 70	249 ± 131	145 ± 65	115 ± 60	224 ± 103
angiotensin II (m/z = 1046.5)	1304 ± 409	1158 ± 323	511 ± 115	1013 ± 725	119 ± 25	875 ± 361
angiotensin I (m/z = 1296.7)	865 ± 248	579 ± 132	339 ± 100	300 ± 127	84 ± 19	265 ± 91
substance P (m/z = 1347.7)	1309 ± 367	431 ± 129	94 ± 17	515 ± 130	48 ± 15	397 ± 140
bombesin (m/z = 1619.8)	387 ± 175	145 ± 72	169 ± 63	621 ± 137	32 ± 11	367 ± 153
renin substrate (m/z = 1758.9)	128 ± 31	105 ± 39	50 ± 14	55 ± 8	21 ± 5	68 ± 20
ACTH clip (1–17) (m/z = 2093.1)	78 ± 38	86 ± 28	9 ± 2	27 ± 9	8 ± 2	37 ± 13
ACTH clip (18–39) (m/z = 2465.2)	6 ± 2	5 ± 1	5 ± 4	9 ± 4	2 ± 1	11 ± 4
somatostatin (m/z = 3147.5)	4 ± 2	5 ± 2	ND	5 ± 3	ND	7 ± 2

[a]Data were acquired on a TOF/TOF and Q-TOF instrument, respectively. [b]SD standard deviation (n = 3).

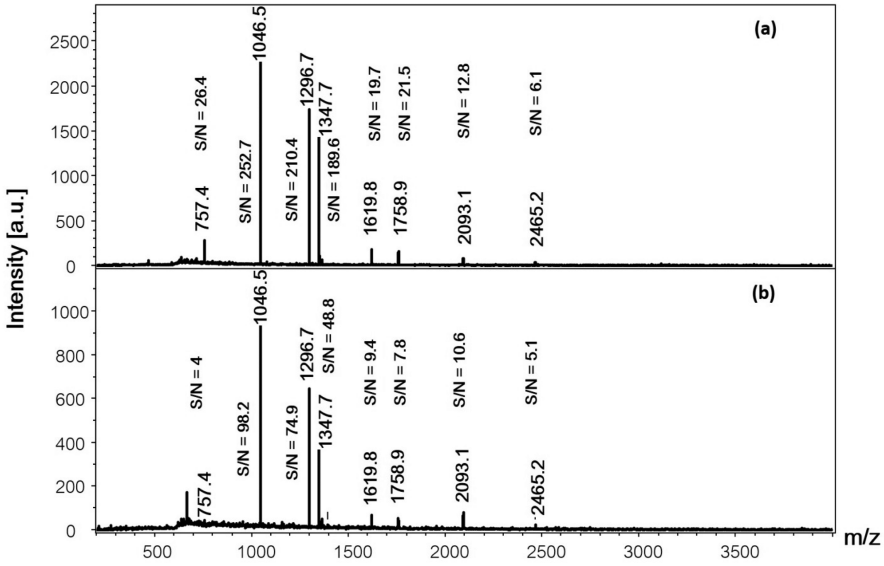


Figure 1. MALDI mass spectra of peptide standards mixture (1 fmol on an AnchorChip target) acquired on an Ultraflex TOF/TOF mass spectrometer using (a) CHCA and (b) ClCCA as matrix and the sample preparation protocol as recommended by the manufacturer. All observed peptide analyte $[M + H]^+$ ions are labeled with their recorded m/z and S/N values.

Biosciences, Morgantown, WV, USA). Mass spectra were acquired on an Ultraflex TOF/TOF mass spectrometer (Bruker UK) in positive ion reflectron mode using a nitrogen laser emitting at 337 nm with a repetition rate of 50 Hz and on a MALDI Q-TOF Premier mass spectrometer (Waters, Wilmslow, UK) in positive ion V mode using a laser emitting at 337 nm with a repetition rate of 20 Hz. For the TOF/TOF data acquisition, each mass spectrum resulted from the accumulation of data acquisitions from 5 different desorption spots with 200 single laser shots each, while for the Q-TOF data acquisition each mass spectrum resulted from the data accumulation of MS scans over 1 min (i.e., 1200 laser shots) at a scan rate of 1 scan per second. Laser fluences on the instruments were optimized in each case for the highest analyte signal-to-noise ratio (S/N).

For the BSA digest analyses, peaks were picked using the SNAP algorithm in the FlexAnalysis software (version 3.0; Bruker UK) with a S/N threshold of 3, a maximum number of 300 peaks, a TopHat baseline subtraction, and "Averagine" for the SNAP average composition. The peak lists were then searched using the peptide mass fingerprint search routine of the search engine Mascot 2.4 (Matrix Science Ltd., London, UK). The search parameters were 100 ppm for peptide mass tolerance, 1 for peptide charge state, trypsin for enzyme, carbamidomethyl (C) for fixed modifications, oxidation (M) for variable modifications, and 1 for max missed cleavages.

Our initial objective was to improve the overall MALDI MS performance by combining AnchorChip targets with the use of the recently introduced matrix ClCCA and thus exploiting their combined increase in peptide ion signal intensity. However, when we used the sample preparation for AnchorChip targets as recommended by the manufacturer (Bruker preparation) for a comparison between the two matrices CHCA and ClCCA, the results showed no advantage using ClCCA. In fact, the average S/N for most of the peptides (7 out of 9) of the peptide standard mixture analyzed at the 20 fmol level was higher for CHCA with some significantly higher ion signals for bradykinin and substance P (see Table 1). Employing a higher ClCCA concentration (5 mg/mL) in the matrix solution or a different mix ratio of analyte to matrix solution (1:1; v/v) as reported for some DD sample preparations using ClCCA and ground stainless steel targets did not result in any substantial improvements (see Figure S-1, Supporting Information). In most cases, these sample preparations actually worsened the analytical performance as one would expect for using sample

DOI: 10.1021/ac504412p
Anal. Chem. 2015, 87, 1485–1488

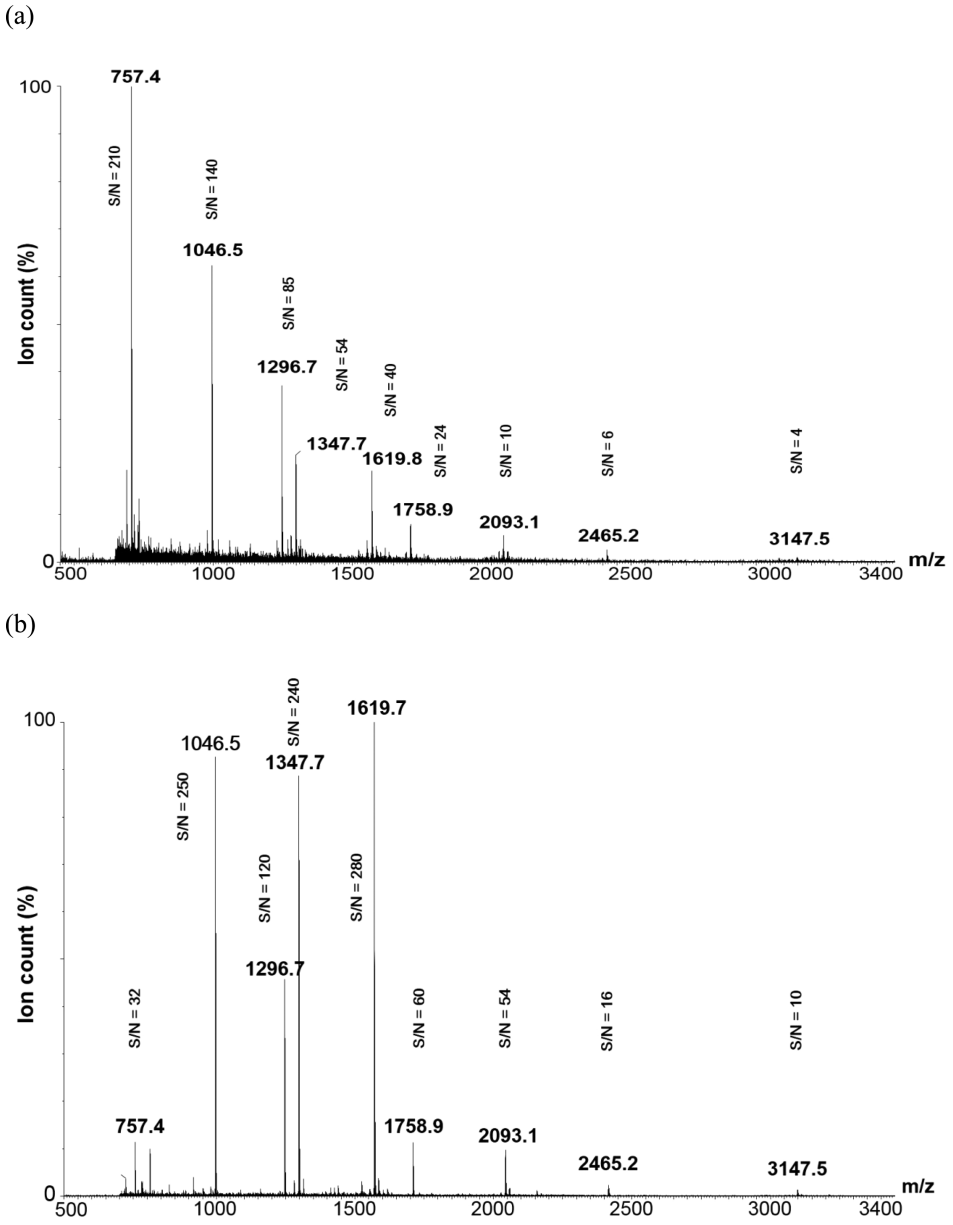


Figure 2. MALDI mass spectra of peptide standards mixture (20 fmol on a stainless steel target) acquired on a Q-TOF Premier mass spectrometer using (a) CHCA (100% intensity = 457 counts) and (b) ClCCA (100% intensity = 1580 counts) as matrix and the sample preparation protocols for each matrix as reported by Leszyk. All observed peptide analyte $[M + H]^+$ ions are labeled with their recorded m/z and S/N values.

preparations that are unconventional for AnchorChip targets. Figure 1 demonstrates the lack of spectral improvement using ClCCA on an AnchorChip target at the lower on-target level of 1 fmol. For most peptides, a higher S/N can be seen again using CHCA compared to ClCCA.

Similar results were obtained with 1 fmol of BSA digest, showing on average (n = 3) a significantly higher Mascot score for CHCA when protein identification was undertaken by peptide mass mapping using the Mascot search engine. Again, S/N of BSA-matched peptides were in general ≥2−3 times higher for CHCA compared to ClCCA samples. This result together with the significantly higher bradykinin ion signal for CHCA was surprising as it was previously reported that ClCCA exhibits a much lower discrimination effect between arginine-containing and other peptides, generally improving the detection of peptides without arginine in mixtures with arginine-containing peptides.

As the results obtained from the AnchorChip targets did not show any improvement in using ClCCA compared to CHCA, we next used ground steel target plates and employed MALDI sample preparations as described in the literature in order to verify earlier reports with respect to the advantages of using ClCCA. These experiments were undertaken on two different mass spectrometers, a Q-TOF instrument with a medium vacuum MALDI ion source and a TOF/TOF instrument with the MALDI ion source under high vacuum. In all cases using the ground steel targets, the sample preparations with ClCCA improved the analyte ion signal-to-noise ratio, also leading to improved protein identification for the analysis of BSA by Mascot peptide mass mapping. Table 1 lists the S/N values for the measurements on both the Ultraflex TOF/TOF and Q-TOF Premier instrument using a stainless steel target. Sample preparations with ClCCA on stainless steel targets clearly outperformed preparations with CHCA, in particular with

DOI: 10.1021/ac504412p
Anal. Chem. 2015, 87, 1485−1488

respect to high molecular mass peptide ions as can be seen in Figure 2.

The results obtained by using stainless steel targets are in good agreement with the literature describing the advantages of using ClCCA as a MALDI matrix. However, to the best of the authors' knowledge, there is only the report by Selman et al.[16] comparing ClCCA with other MALDI matrices on AnchorChip targets or other targets that generally improve MALDI MS sensitivity using CHCA as matrix. Interestingly, this report focuses on the comparison with DHB, a matrix that does not benefit as much as CHCA from the use of AnchorChip targets, and on the analysis of glycans and glycopeptides, an analyte/peptide subset known to be difficult to analyze using the "hot" matrix CHCA. Selman et al. predominately used normal stainless steel target plates for the ClCCA-based samples in their comparisons but AnchorChip targets for other matrices, suggesting that also in their study AnchorChip targets did not improve analytical sensitivity using ClCCA. Thus, despite the well-demonstrated increase in analytical sensitivity by using ClCCA as matrix in MALDI samples on stainless steel targets, the same was not found for AnchorChip targets when compared to CHCA. Although we have employed various sample preparations with the use of AnchorChip targets, we were not able to find a sample preparation that led to better analytical sensitivity using ClCCA compared to CHCA (using the manufacturer's recommended sample preparation protocol). It obviously remains to be seen whether an improved ClCCA-specific sample preparation for the use of AnchorChip targets can be found that will lead to a similar comparative advance in analytical sensitivity as can be seen with stainless steel targets.

In summary, it can be concluded that with the introduction of newly designed matrix systems, showing increased (sometimes "best") analytical performance for a given set of experiments, a wider set of parameters needs to be investigated before a new matrix system can be classified as an improvement to currently available MALDI matrix systems. For ClCCA, it appears to be true that it easily outperforms the established matrices (e.g., CHCA) if prepared with the dried droplet sample preparation method on normal stainless steel targets. However, when prepared using more sophisticated sample preparation methods (e.g., using AnchorChip targets), which have been shown to further improve analytical performance substantially for the well-established matrices, ClCCA's performance does not necessarily improve as much, losing all the comparative advantage to other matrices that has previously been reported under certain conditions.

Our results are a stark reminder that for all reports of new MALDI matrices and matrix systems the utmost care needs to be employed in concluding that a new matrix (as well as matrix system, sample preparation, etc.) is a true advancement in analytical performance. Ideally, as many performance-relevant parameters as possible should be investigated with at least two mandatory sets of experiments: (i) direct comparison of the analytical performance using the respectively best method (including sample preparation and data acquisition parameters) for both the newly introduced system and the currently best system available and (ii) a comparison with benchmark data at the absolute level (e.g., absolute limits of detection and absolute ion intensity levels) using all available sources, including data acquired outside the inventor's lab (e.g., literature data). Finally, in the context of the latter, it should become compulsory that any record-breaking limits of detection, particularly beyond the femtomole level, should only be acceptable if the measurements have been performed on new (previously unused) targets and/or analytes that are not normally used in the lab. These measures would significantly reduce the influence of residual analyte material from previous experiments on the reported analyte ion signal intensity, thus providing a much greater confidence in the reported limit of detection.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary mass spectra (Figure S-1) as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Fax:+44 118 378 6331. Tel: +44 118 378 4550. E-mail: r.k.cramer@reading.ac.uk.

Author Contributions

The manuscript was written through contributions of both authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Royal Thai Government (K.W.). We thank the Chemical Analysis Facility at the University of Reading for access to the Ultraflex mass spectrometer.

■ REFERENCES

(1) Karas, M.; Bachmann, D.; Bahr, U.; Hillenkamp, F. *Int. J. Mass Spectrom. Ion Processes* **1987**, *78*, 53−68.
(2) Armstrong, D. W.; Zhang, L. K.; He, L. F.; Gross, M. L. *Anal. Chem.* **2001**, *73*, 3679−3686.
(3) Cramer, R.; Corless, S. *Proteomics* **2005**, *5*, 360−370.
(4) Cramer, R.; Pirkl, A.; Hillenkamp, F.; Dreisewerd, K. *Angew. Chem., Int. Ed. Engl.* **2013**, *52*, 2364−2367.
(5) Kolli, V. S. K.; Orlando, R. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 923−926.
(6) Tholey, A.; Heinzle, E. *Anal. Bioanal. Chem.* **2006**, *386*, 24−37.
(7) Towers, M.; Cramer, R. *Spectroscopy* **2007**, *22*, 29−37.
(8) Towers, M. W.; McKendrick, J. E.; Cramer, R. *J. Proteome Res.* **2010**, *9*, 1931−1940.
(9) Crank, J. A.; Armstrong, D. W. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1790−1800.
(10) Cramer, R.; Karas, M.; Jaskolla, T. W. *Anal. Chem.* **2014**, *86*, 744−751.
(11) Jaskolla, T.; Fuchs, B.; Karas, M.; Schiller, J. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 867−874.
(12) Jaskolla, T. W.; Lehmann, W. D.; Karas, M. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 12200−12205.
(13) Soltwisch, J.; Jaskolla, T. W.; Hillenkamp, F.; Karas, M.; Dreisewerd, K. *Anal. Chem.* **2012**, *84*, 6567−6576.
(14) Palmblad, M.; Cramer, R. *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 693−697.
(15) Leszyk, J. D. *J. Biomol. Tech.* **2010**, *21*, 81−91.
(16) Selman, M. H.; Hoffmann, M.; Zauner, G.; McDonnell, L. A.; Balog, C. I.; Rapp, E.; Deelder, A. M.; Wuhrer, M. *Proteomics* **2012**, *12*, 1337−1348.

DOI: 10.1021/ac504412p
Anal. Chem. 2015, 87, 1485−1488