Analytical Methods

RSC Publishing

TECHNICAL NOTE

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2013, **5**, 4258

Compatible buffer for capillary electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry

Hong-Xu Chen,[ab] Jean-Marc Busnel,[a] Liang Qiao,[a] Natalia Gasilova,[a] Xin-Xiang Zhang*[b] and Hubert H. Girault*[a]

Received 11th March 2013
Accepted 3rd June 2013

DOI: 10.1039/c3ay40397a

www.rsc.org/methods

A compatible buffer system for coupling of capillary electrophoresis (CE) with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was developed. The employed interface consists of a robot to drive a silver-covered separation capillary and an AnchorChip MALDI-MS target. The outlet of the capillary is grounded and connected to the pre-deposited buffer droplet on the MALDI target to make the electric connection and allow sample crystallization for MALDI-MS. The possibility of using only one buffer already containing the matrix for MALDI-MS for separation and ionization was investigated and tested on protein and peptide samples. The results show that the proposed buffer system is suitable for CE-MALDI-MS coupling, simplifies the traditional buffer mixing steps in off-line CE-MALDI-MS protocols, and is therefore highly promising for on-line analysis.

1 Introduction

Proteomics has been proven to be a very challenging task. From an analytical standpoint, proteomics is much more complex than genomics. The difficulties in proteomics come from the various post-translational modifications, the large dynamic range of proteins and the temporal variations. To date, one of the most widely used strategies in proteomics has been the shotgun proteomics, where proteins are digested into peptides, separated by liquid chromatography (LC) and identified by tandem mass spectrometry (MS/MS).[1]

As another important separation technique, capillary electrophoresis holds the advantage of high efficiency, simple instrumentation, rapid separation and low sample consumption.[2] The coupling of capillary electrophoresis (CE) with mass spectrometry (MS) was initially developed by Smith *et al.* in the 1980s,[3] and is now a rather powerful analytical technique. The coupling of CE with MS is mainly realized for electrospray ionization mass spectrometers (ESI-MSs). Different interfaces for CE-ESI-MS coupling have been developed, including sheath-flow interfaces, sheathless interfaces and liquid junction interfaces. A comprehensive review on CE-ESI-MS has been recently published.[2] Commercial interfaces are available from Agilent and Beckman Coulter for coupling CE with ESI-MS.

Matrix-assisted laser desorption/ionization (MALDI) is another soft ionization technique employed in MS for protein identification. Unlike ESI, which is a continuous liquid introduction technique, MALDI has been mainly used for the analysis of solid samples. Compared to ESI, MALDI supplies higher sensitivity and tolerance to salts and buffers. Thus, the coupling of CE and MALDI-MS has also drawn great interest.[4,5] Several approaches for coupling CE and MALDI-MS have been described, where the fraction was collected from the eluting stream and spotted into wells or directly onto the target plate with the aid of liquid sheath flow.[6–10] Additional interface designs for coupling CE and MALDI-MS include rotating ball inlet,[11] vacuum deposit,[12,13] porous polymer joint,[14] inkjet technology,[15] droplet electrocoupling[16] and so on. An alternative CE-MALDI-MS coupling strategy is an iontophoretic spotting approach, with the outer outlet part of the separation capillary being coated with silver based conducting paste.[17–19] Previously, matrix solution was introduced by mixing with the spotted sample at the outlet terminal of the capillary by an additional step after fraction collection or before sample introduction. Furthermore, a desalting step was needed in some cases, when the chosen CE buffer contains a large amount of salts hindering the MALDI-MS detection.

Here, a compatible solution (separation buffer), 4% formic acid (FA) with 1.25 mg mL^{-1} 2,5-dihydroxybenzoic acid (DHB), for both CE separation and MALDI-MS was investigated. CE and MALDI were coupled using the iontophoretic spotting approach mentioned above. 10 μL of the chosen separation buffer was deposited in advance on the hydrophobic target with a hydrophilic anchor to concentrate the separated fraction. This coupling system allowed the collection of the CE fraction directly on the MALDI-MS target without the breakdown of the current. It was observed that the addition of DHB as a MALDI

[a]*Laboratoire d'Electrochimie Physique et Analytique, EPFL SB ISIC LEPA, Station 6, CH-1015, Lausanne, Switzerland. E-mail: hubert.girault@epfl.ch*

[b]*Beijing National Laboratory for Molecular Sciences, College of Chemistry, Peking University, Beijing, 100871, China. E-mail: zxx@pku.edu.cn*

matrix to a background electrolyte (BGE) did not disturb the CE separation causing only small losses of the UV absorption signal. Meanwhile the use of FA as a separation buffer prevents the addition of acetonitrile (ACN) and trifluoroacetic acid (TFA) into the DHB matrix. The described system allows elimination of the buffer mixing and desalting steps in the current procedure of coupling CE and MALDI-MS.

2 Materials and methods

2.1 Chemicals

All chemicals used, such as β-lactoglobulin, α-lactoalbumin, ribonuclease S and 2,5-dihydroxybenzoic acid (DHB), were analytical reagent grade and obtained from Sigma-Aldrich (Schnelldorf, Switzerland). All solvents used for CE, MALDI-MS such as formic acid, acetonitrile, trifluoroacetic acid, acetone, methanol, hexane, and isopropanol were analytical reagent grade and obtained from Sigma-Aldrich (Schnelldorf, Switzerland). Peptides were obtained by digesting the corresponding proteins with trypsin following the standard in-solution digestion protocol. External calibration for MALDI-TOF (time of flight)-MS was obtained from Bruker (Bremen, Germany) containing angiotensin I (1296.685 Da) and II (1046.542 Da), substance P (1347.735 Da), bombesin (1619.822 Da) and ACTH_Clip (18–39) (2465.198 Da). All buffer and sample solutions were prepared with water produced using an alpha Q Millipore system (Zug, Switzerland).

2.2 Capillary electrophoresis

CE-UV experiments for buffer and additive optimization were carried out on a P/ACE MDQ system (Beckman, Brea, USA). CE-MS experiments were performed on a HP^{3D}CE apparatus (Agilent, Waldbronn, Germany). Fused-silica capillaries (50 μm i.d., 375 μm o.d.) were obtained from BGB Analytik AG (Böckten, Switzerland). The separation capillary was conditioned by sequentially rinsing (pressure, 20 psi) with 1 M sodium hydroxide for 10 min, deionized water for 10 min and running buffer for 5 min. Between consecutive analyses, the capillary was flushed with 1 M sodium hydroxide, distilled water and running buffer in turn (2 min for each).

2.3 CE-MALDI MS interface

15 cm of one extremity of the capillary was painted with silver ink from Ercon (Wareham, MA, USA). Then the capillary was held at 80 °C for two hours in an oven. The layout of the whole setup was the same as the previous report.[17–19] Briefly, while the non-painted terminal was in the CE apparatus, the painted one was placed in a ceramic holder, being an integrated part of a home-made robotic system able to move in three axes controlled *via* a home developed program (Labview, National Instruments, Austin, TX, USA). A 96-well Anchor target plate was placed on the moving stage of the robotic system. The painted terminal of the capillary was grounded and dipped into the pre-deposited droplet on the target to form the electro-connection for CE. As the target moves along the *x* and *y*-axes and the capillary along the *z*-axis, different fractions could be spotted onto the target. The spotting time for each fraction could be easily set *via* the controlling program.

2.4 MALDI-TOF-MS

All MALDI-TOF experiments have been carried out on a Microflex LRF instrument (Bruker Daltonics, Bremen, Germany) equipped with a nitrogen laser operating at 337 nm. Positively charged ions were detected and the sum of 400 single spectra was used for data treatment. 10 μL of 4% FA with 1.25 mg mL^{-1} DHB was deposited before the separation step on each spot of the AnchorChip target plate (Bruker). This volume was high enough to keep the resulting droplets on the plate during the time required for the separation (≈45 min) in spite of the evaporation. After the collection of the separated proteins or peptides, the droplets were allowed to dry at room temperature under atmospheric pressure to crystallize the sample within the matrix. The AnchorChip was washed each time after the experiment as follows: washing with acetone, ultrasonication in methanol/water for 30 min, and washing again with acetone, hexane and isopropanol.

3 Results and discussion

3.1 Optimization of CE buffer for protein separation

Formic acid was chosen as the BGE for the CE separation of proteins and peptides. A higher concentration of formic acid gave better resolution for proteins (Fig. 1A), and a similar result was also obtained for peptides (Fig. 1B). The increased FA amount decreased the pH and minimized the ionization of the capillary surface and the adsorption of the samples, thus increasing the resolution of protein and peptide separation. The vial containing the BGE buffer was changed to a new one every 10 runs in order to keep the pH of the BGE constant during all the experiments. The relative standard deviations (RSDs) of the migration time for the six proteins in Fig. 1A are from 0.2% to 1.6% ($n = 3$).

Since the use of FA has a potential influence on MALDI-MS, the choice of its concentration should compromise the separation and MS ionization efficiency. 4% of FA in water was

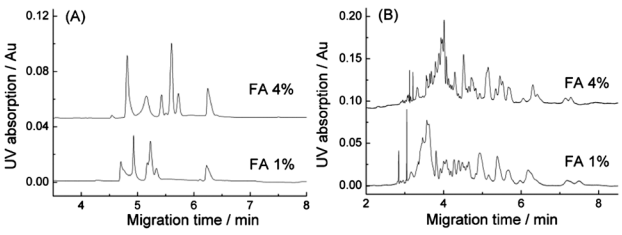

Fig. 1 Separation of proteins (A) and peptides (B) with 1% and 4% of formic acid in water. (A) Conditions: capillary: uncoated and activated with NaOH, 50/40 cm total/effective length with 50 μm i.d.; electrokinetic injection: 1 kV for 40 s; applied voltage: 15 kV; detection: 200 nm UV; sample: lysozyme (125 μg mL^{-1}), BSA (92 μg mL^{-1}), α-LA (104 μg mL^{-1}), RNA (158 μg mL^{-1}), RNS (42 μg mL^{-1}) and bradykinin (83 μg mL^{-1}) in water. (B) Conditions: capillary: uncoated and activated with NaOH, 50/41.5 cm total/effective length with 50 μm i.d.; hydrodynamic injection: 30 mbar for 120 s; applied voltage: 25.5 kV; detection: 200 nm UV; sample: mixture of the digested BSA and RNA, 0.5 mg mL^{-1} for each.

finally used as the BGE. As proved by the later experiment, the sensitivity of MALDI-TOF-MS was well kept with this concentration of FA.

3.2 MALDI matrix as an additive in CE separation

In order to understand whether DHB disturbs the CE separation, DHB with different concentrations (0.31, 0.63, 1.25, 2.50 to 5 mg mL^{-1}) was added into the BGE (FA = 4%) for the CE separation of proteins. As the concentration of DHB increased, the peak heights of the separated proteins decreased and disappeared at the concentration above 2.5 mg mL^{-1}, due to the strong UV absorption of DHB present in BGE (Fig. 2). However, the migration times of all the proteins were kept the same. These results suggest that the addition of DHB does not influence the CE separation except for UV detection sensitivity. When MS is used for the analyte detection, it is possible to apply the DHB as an additive in BGE for the CE separation.

3.3 Effect of CE BGE in MALDI-MS

Results presented above show that DHB could be added to the CE buffer; the next step is to study whether the chosen BGE with FA is compatible with MALDI-MS analysis. The test was carried out by determining the limits of detection (LODs) of three proteins in both a traditional MALDI matrix (10 mg mL^{-1} DHB in 50% ACN/49.9% water/0.1% TFA) and the modified CE buffer (1.25 mg mL^{-1} DHB in 4% FA/96%water). The AnchorChip with a hydrophilic spot in the hydrophobic surface was used to concentrate the sample. Due to the strong hydrophilicity of the modified BGE, 10 μL of it can be deposited onto the hydrophilic spot on the target without diffusion and contamination between neighbouring spots. In contrast, only 1 μL of sample in traditional matrix solution was deposited on the AnchorChip spot because of the large presence of ACN. Assuming similar LODs in both buffer systems, the detectable sample concentration in the former can be ten times lower than that in the latter. The results presented in Table 1 showed that the LODs in two buffers were at the same levels for β-LG and α-LA. For RNS, the LOD in the modified CE buffer was even lower than that in the traditional MALDI matrix.

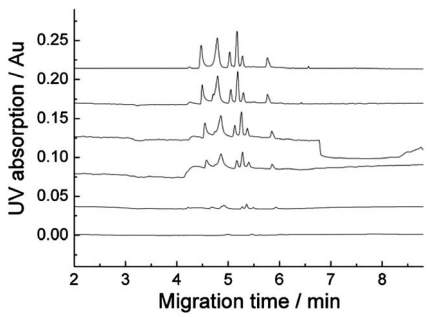

Fig. 2 Effect of DHB concentrations in CE separation. BGE: 4% of formic acid in water with a DHB concentration of 0, 0.31, 0.63, 1.25, 2.50 and 5 mg mL^{-1} (from top to bottom). Other conditions are the same as those in Fig. 1A.

Table 1 LODs of three proteins in two buffers on the AnchorChip target plate

Buffer	β-LG	α-LA	RNS
Buffer-1[a] (1 μL)	38 fmol	12 fmol	1.4 fmol
Buffer-2[b] (10 μL)	42 fmol	14 fmol	173 amol

[a] 10 mg mL^{-1} DHB in 50% ACN/49.9% water/0.1% TFA. [b] 1.25 mg mL^{-1} DHB in 4% FA%/96% water.

3.4 Off-line combination of CE-MALDI-MS for proteins and peptides

The off-line combination of CE and MALDI-MS using the reported iontophoretic spotting approach[17] with the proposed compatible buffer was investigated by separating and detecting a protein mixture containing β-LG, α-LA and RNS. Serial decreasing of the injection amount was tested. With the highest injection amount, the same protein appeared in two different fractions due to the peak broadening as a consequence of sample overloading (Table 2A). As the injection amount decreased, the three proteins were well separated into different fractions (Table 2B). In the case of the lowest injection amount, β-LG cannot be detected (Table 2C). Such a result was reasonable since the above-mentioned LOD of β-LG on MALDI-MS was higher than those of the other two proteins. Compared with the theoretical injection amounts of β-LG (84 fmol in Table 2C), the recovery of the whole off-line CE-MALDI-MS was acceptable. Fig. 3 shows the mass spectra of different fractions obtained under various sample injection amounts.

Digested peptides from BSA and RNA were also tested with the proposed compatible separation buffer by the off-line CE-MALDI-MS analysis. The number of matched peptides for each protein was counted in order to estimate the efficiency of the method. In comparison with the data from direct MALDI-MS detection using a traditional matrix, the number of matched peptides of BSA from CE-MALDI-MS was the same when each fraction was collected during 60 s of CE separation. Meanwhile, the number of matched peptides for RNA increased from 4 to 6, indicating that the addition of the separation step benefited the identification of peptides *via* decreasing the ion suppression effect. Indeed, RNA is a glycosylated protein. It is hard to be digested by trypsin and analysed under the standard MALDI-MS condition, which explains the number of peptides identified. Moreover, when the stop time for each fraction decreased from

Table 2 Fractions from off-line CE-MALDI-MS. Hydrodynamic injection: 30 mbar for (A) 80 s, (B) 40 s and (C) 20 s. Sample: β-LG (125 μg mL^{-1}), α-LA (208 μg mL^{-1}), and RNS (50 μg mL^{-1}) in water. CE conditions: capillary: 85 cm with 50 μm i.d., uncoated and activated with NaOH; applied voltage: 25.5 kV; isotachophoresis (ITP): NC (250 mM, pH = 4) 30 mbar for 40 s. MALDI-MS fraction: 45 s for each fraction with 10 μL compatible buffer on the plate

Fraction	A	B	C
6	—	β-LG	—
7	β-LG	—	—
8	α-LA	α-LA	α-LA
9	α-LA	—	—
10	RNS	RNS	RNS
11	RNS	—	—

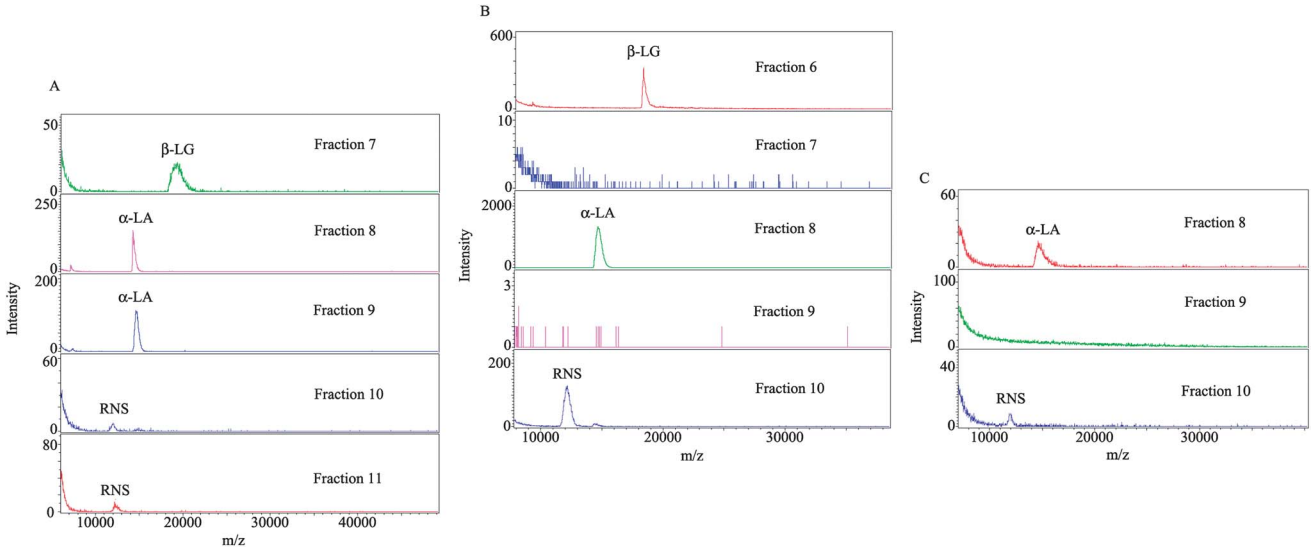

Fig. 3 The mass spectra of different fractions in Table 2 obtained for various sample injection amounts.

Table 3 Number of matched peptides of BSA and RNA with compatible separation buffer. For direct MALDI-MS, 1 μL of the digested sample in a traditional matrix (574 fmol of BSA and 3.32 pmol of RNA) was deposited onto the target. For CE-MALDI-MS, the digested sample (746 fmol of BSA and 4.3 pmol of RNA) was injected and each MALDI fraction was collected during 60 or 30 s of CE separation, respectively. CE conditions are the same as those in Table 2 with 80 s injection

	BSA	RNA
Direct MALDI-MS	22	4
CE-MALDI-MS/60 s fraction	22	6
CE-MALDI-MS/30 s fraction	29	6

60 to 30 s, more fractions were collected for MALDI-MS analysis, and more peptides of BSA were identified (Table 3).

4 Conclusion

A separation buffer system of 1.25 mg mL^{-1} DHB in 4% of FA was used for off-line CE-MALDI-MS analysis of proteins and peptides. As the addition of DHB just caused a decrease of the UV absorption but did not disturb the separation efficiency, it can be used directly in CE separation followed by MS detection. Moreover, this modified BGE offered comparable results with the traditional MALDI-MS matrix. Due to the enrichment effect on the AnchorChip, the sensitivity of sub fmols for protein detection was obtained, with satisfactory sample recovery. The chosen separation buffer was compatible with both CE and MALDI-MS, which could simplify the off-line combination of these two techniques. The possibility of applying the proposed BGE in an off-line CE-MALDI-MS was investigated by separating and identifying the protein mixture and peptides generated by protein tryptic digestion. The obtained results show that the BGE containing a MALDI matrix provided better sample identification than direct MALDI-MS by decreasing the ion suppression effect. Proposed separation buffer could be also promising to the on-line CE-MALDI-MS analysis of peptides and proteins.

Acknowledgements

Supported by a grant from the European Community (Contract no. 12793) project "Allergy Card". H. X. Chen and X. X. Zhang are grateful to National Natural Science Foundation of China (no. 90713013, 30890142, 20975007) and the National Scientific Support Project (no. 2009CB320305, MOST, China).

Notes and references

1 D. A. Wolters, M. P. Washburn and J. R. Yates, *Anal. Chem.*, 2001, **73**, 5683–5690.
2 G. Bonvin, J. Schappler and S. Rudaz, *J. Chromatogr., A*, 2012, **1267**, 17–31.
3 J. A. Olivares, N. T. Nguyen, C. R. Yonker and R. D. Smith, *Anal. Chem.*, 1987, **59**, 1230–1232.
4 R. Haselberg, G. J. de Jong and G. W. Somsen, *J. Chromatogr., A*, 2007, **1159**, 81–109.
5 C. W. Huck, R. Bakry, L. A. Huber and G. K. Bonn, *Electrophoresis*, 2006, **27**, 2063–2074.
6 T. Johnson, J. Bergquist, R. Ekman, E. Nordhoff, M. Schurenberg, K. D. Kloppel, M. Muller, H. Lehrach and J. Gobom, *Anal. Chem.*, 2001, **73**, 1670–1675.
7 M. Lechner, A. Seifner and A. M. Rizzi, *Electrophoresis*, 2008, **29**, 1974–1984.
8 A. Chartogne, M. Gaspari, S. Jespersen, B. Buscher, E. Verheij, R. van der Heijden, U. Tjaden and J. van der Greef, *Rapid Commun. Mass Spectrom.*, 2002, **16**, 201–207.
9 J. S. Page, S. S. Rubakhin and J. V. Sweedler, *Anal. Chem.*, 2002, **74**, 497–503.
10 W. J. Yu, Y. Li, C. H. Deng and X. M. Zhang, *Electrophoresis*, 2006, **27**, 2100–2110.
11 H. Orsnes, T. Graf, H. Degn and K. K. Murray, *Anal. Chem.*, 2000, **72**, 251–254.

12 J. Preisler, P. Hu, T. Rejtar and B. L. Karger, *Anal. Chem.*, 2000, **72**, 4785–4795.
13 J. Preisler, P. Hu, T. Rejtar, E. Moskovets and B. L. Karger, *Anal. Chem.*, 2002, **74**, 17–25.
14 J. Wang, M. Ma, R. Chen and L. Li, *Anal. Chem.*, 2008, **80**, 6168–6177.
15 M. W. Vannatta, C. D. Whitmore and N. J. Dovichi, *Electrophoresis*, 2009, **30**, 4071–4074.
16 N. Ojima, T. Shingaki, T. Yamamoto and T. Masujima, *Electrophoresis*, 2001, **22**, 3478–3482.
17 J.-M. Busnel, J. Josserand, N. Lion and H. H. Girault, *Anal. Chem.*, 2009, **81**, 3867–3872.
18 M. R. Pourhaghighi, J.-M. Busnel and H. H. Girault, *Electrophoresis*, 2011, **32**, 1795–1803.
19 N. Gasilova, A.-L. Gassner and H. H. Girault, *Electrophoresis*, 2012, **33**, 2390–2398.

