Supporting Information


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Fig. S-1. Effects of MDPNA matrix additive for CHCA on ionization suppression caused by inorganic salts. The seven-peptide mix was diluted with 50 mM NaCl or 50 mM Na$_2$PO$_4$, pH 9.0, at concentrations of 300 fmol/μL each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH 1-17, and ACTH 18-39, and 200 fmol/μL of ACTH 7-38. A portion (0.5 μL) of the seven-peptide mix in the inorganic salt solution, 0.5 μL of matrix CHCA, and 0.5 μL of MDPNA solution (with MDPNA) or Milli-Q water (without MDPNA) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum.
Fig. S-2. Effects of MDPNA matrix additive for DHB on ionization suppression caused by inorganic salts. The seven-peptide mix was diluted with 50 mM NaCl or 50 mM Na$_2$PO$_4$, pH 9.0, at concentrations of 300 fmol/μL each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH1-17, and ACTH18-39, and 200 fmol/μL of ACTH7-38. A portion (0.5 μL) of the seven-peptide mix in the inorganic salt solution, 0.5 μL of matrix DHB, and 0.5 μL of MDPNA solution (with MDPNA) or Milli-Q water (without MDPNA) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum.
Fig. S-3. Effect of adding MDPNA to the CHCA matrix in negative ion mode. Data for each peptide were analyzed by a Mann–Whitney U test. Asterisks (*) represent statistically significant differences (n = 8). The seven-peptide mix (150 fmol each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH 1-17, and ACTH 18-39, and 100 fmol of ACTH7-38) in (a) 0.1% TFA not containing buffering compounds, (b) pH 4.0 amine buffer, or (c) pH 8.0 amine buffer (matrix CHCA with or without MDPNA) was analyzed by MALDI-TOFMS. The analytical conditions were negative ion linear mode with 500 laser shots for one spectrum.
Fig. S-4. Effect of adding MDPNA to the DHB matrix in negative ion mode. Data for each peptide were analyzed by a Mann–Whitney U test. Asterisks (*p < 0.05) represent statistically significant differences (n = 8). The seven-peptide mix (150 fmol each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH 1-17, and ACTH 18-39, and 100 fmol of ACTH7-38) in (a) 0.1% TFA not containing buffering compounds, (b) pH 4.0 amine buffer, or (c) pH 8.0 amine buffer (matrix DHB with or without MDPNA) was analyzed by MALDI-TOFMS. The analytical conditions were negative ion linear mode with 500 laser shots for one spectrum.
Fig. S-5. Structures and abbreviations of candidate additives.
Fig. S-6. Signal-to-noise (S/N) ratios in MALDI-MS analyses using the DHB matrix and additives (MDPNA, PAC, EDPNA, and HEPD). The seven-peptide mix was diluted with (a) 0.1% TFA (not containing buffering compounds) or (b, c, d, e, f) pH 8.0 amine buffer at concentrations of 300 fmol/μL each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH 1-17, and ACTH 18-39, and 200 fmol/μL of ACTH 7-38. A portion (0.5 μL) of the seven-peptide mix in pH 8.0 amine buffer or 0.1% TFA, 0.5 μL of matrix DHB, and 0.5 μL of (c) 1% MDPNA, (d) PAC, (e) EDPNA, (f) HEDP or (a, b) Milli-Q water (without additive) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum. The data for each peptide were analyzed by a Kruskal–Wallis H test followed by a Mann–Whitney U test with Benjamini-Hochberg correction as a post-hoc test. Asterisks (*p < 0.05) represent statistically significant differences.
Fig. S-7. Signal-to-noise (S/N) ratios in MALDI-MS analyses using the DHB matrix and additives (1% MDPNA, 1% TFA, 3% TFA, 10 mM DAC, and 1% PA). The seven-peptide mix was diluted with pH 8.0 amine buffer at concentrations of 300 fmol/μL each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH 1-17, and ACTH 18-39, and 200 fmol/μL of ACTH 7-38. A portion (0.5 μL) of the seven-peptide mix in pH 8.0 amine buffer, 0.5 μL of matrix DHB, and 0.5 μL of additive (1% MDPNA, 1% TFA, 3% TFA, 10 mM DAC, or 1% PA) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum. The data for each peptide were analyzed by a Kruskal–Wallis H test followed by a Mann–Whitney U test with Benjamini-Hochberg correction as a post-hoc test. Asterisks (*) p < 0.05) represent statistically significant differences.
Fig. S-8. Effect of adding MDPNA to the CHCA matrix on ionization suppression by pH 8.0, pH 6.0, and pH 4.0 amine buffers. Data for each peptide were analyzed by a Mann–Whitney U test. Asterisks (*$p < 0.05$) represent statistically significant differences ($n = 8$). The seven-peptide mix (150 fmol each of Ang I, Ang II, [Glu1]-Fib, N-Acetyl-Renin, ACTH 1-17, and ACTH 18-39, and 100 fmol of ACTH7-38) in amine buffer (pH 8.0, 6.0, or 4.0) was analyzed by MALDI-TOFMS (matrix CHCA with or without MDPNA). The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum.
Fig. S-9. Comparison of mass spectra of the BSA tryptic digest with or without MDPNA (Matrix: DHB). The protein digestion standard BSA was diluted with (a) 0.1% TFA not containing buffering compounds, (b) pH 6.0 amine buffer, or (c) pH 8.0 amine buffer at concentrations of 100 fmol/μL. A portion (0.5 μL) of the tryptic digest in the amine buffer (pH 8.0 or 6.0) or 0.1% TFA, matrix DHB, and 1% MDPNA (with MDPNA) or Milli-Q water (without MDPNA) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum.
Fig. S-10. Comparison of sequence coverage ratios with and without MDPNA after MALDI-MS peptide mass-fingerprinting analysis. The protein digestion standard BSA was diluted with 0.1% TFA not containing buffering compounds, pH 6.0 amine buffer, or pH 8.0 amine buffer at concentrations of 100 fmol/μL. A portion (0.5 μL) of the tryptic digest in the amine buffer (pH 8.0 or 6.0) or 0.1% TFA, matrix CHCA, and 1% MDPNA (with MDPNA) or Milli-Q water (without MDPNA) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum. Data for each peptide were analyzed by a Kruskal–Wallis H test followed by a Mann–Whitney U test with Benjamini–Hochberg correction as a post-hoc test. Asterisks (*p < 0.05) represent statistically significant differences. (means ± S.D., n = 6)
Fig. S-11. Comparison of mass spectra of the BSA tryptic digest with or without MDPNA (Matrix: CHCA). The protein digestion standard BSA was diluted with (a) 0.1% TFA not containing buffering compounds, (b) pH 6.0 amine buffer, or (c) pH 8.0 amine buffer at concentrations of 100 fmol/μL. A portion (0.5 μL) of the tryptic digest in the amine buffer (pH 8.0 or 6.0) or 0.1% TFA, matrix CHCA, and 1% MDPNA (with MDPNA) or Milli-Q water (without MDPNA) were mixed on a MALDI target plate and analyzed by MALDI-TOFMS after drying. The analytical conditions were positive ion linear mode with 500 laser shots for one spectrum.
Fig. S-12. MALDI-MS/MS spectra of tryptic fragments of BSA, a) YLYEIAR, and b) LGEYGFQNALIVR after separation by zwitterionic HILIC.
Fig. S-13. MALDI-MS/MS spectra of (a) [Glu1]-Fib and (b) ACTH18-39 after separation by chromatofocusing.
Fig. S-14. Quasi-linear pH gradient on a DEAE column, Inertsil AX, 5 μm, 0.5 × 150 mm.
Initial buffer, 100 mM Tris-HCl, pH 8.0; elution buffer, 35 mM Tris-HCl, 0.625 mM Bis-Tris, 0.625 mM imidazole, 0.625 mM piperazine, 0.4375 mM N-methylpiperazine, pH 3.3; flow rate, 8.0 μL/min. The chromatofocusing conditions were optimized based on Bates and Frey’s method.1)

REFERENCE