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Matrix with High Salt Tolerance for the Analysis of Peptide and Protein Samples by Desorption/ Ionization Time-of-Flight Mass Spectrometry

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High concentrations of urea and guanidine hydrochloride are commonly used for the denaturation of protein, which was digested by enzymatic proteolysis for the identification by MS analysis. The presence of these contaminants seriously suppresses the ion signal of analytes in MALDI-TOF MS analysis. Herein, a novel MALDI matrix, 3, 4-diaminobenzophenone (DABP), has been found with high tolerance for these contaminants in MALDI MS analysis. The ion signal of analyte insulin can be detected in the presence of 2 M guanidine hydrochloride and 1.5 M urea using DABP as matrix. The tryptic digest of BSA (400 fmol) in 1 M guanidine hydrochloride or 1 M urea was successfully analyzed without any pretreatment prior to MS analysis. Furthermore, it has been found that this matrix can also effectively suppress the cation ion adduction of the peptides in the presence of high concentrations of metal ions in sample solution.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is becoming a powerful tool for the proteome analysis.[1−3] Despite the increased knowledge of the MALDI process, the selection of a suitable matrix is still an empirical procedure. Some benzoic acid and cinnamic acid derivatives, particularly 2,5-dihydroxybenzoicacid (DHB) and α-cyano-4-hydroxycinnamic acid (CHCA), have been proved to be good MALDI matrixes for the analysis of peptides and proteins.[4−5] A large number of new compound candidates have been evaluated as potential MALDI matrixes for this purpose, and some applications of new matrixes have been reported, mainly by trial and error.[6−13] Furthermore, a variety of inorganic materials, such as porous silicon, graphite particles, carbon nanotubes, and active carbon, have also all been evaluated as MALDI matrixes with different degrees of success.[14−19] The most prominent work in this field was reported by Wei and co-workers, in which the porous silicon surface freshly prepared by electrochemical etching was used as MALDI matrix to analyze small molecules, peptides, and polymers.[14] Recently, the oxidized carbon nanotubes also showed good potential as a matrix to analyze small molecules by MALDI MS.[18]

MALDI MS can usually meet the requirements for the analysis of peptides, proteins, and oligonucleotides using an organic matrix; however, some practical problems still exist in the application of this popular technique. For example, urea or guanidine hydrochloride is the most often used denaturant for the proteins and is also used to prevent aggregation and precipitation of hydrophobic proteins. Although MALDI-TOF MS with a conventional matrix has a tolerance for a low concentration of these contaminants.[20,21] residual amounts of those compounds will decrease or suppress the ion signal of analytes[22] and thereby seriously degrades the quality of mass spectra. When the salt concentration reaches ∼1 M, analyte signals are usually undetectable.[21] To solve the contamination problem of urea, guanidine, and other contaminants and improve the performance of mass spectrometry analysis, a number of methods for the sample pretreatment such as HPLC,[23] C18 Ziptip,[24] microextraction chip,[25,26] sample preparation tech-

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niques,[27,28] and polymeric membranes[29−33] have been developed prior to MALDI MS analysis. The removal of contaminants and the decrease of metal cation adducts by these common methods is effective, but the poor recovery of particular peptides in low abundance and sample loss has been observed.[34] Other drawbacks of these methods are time-consuming, labor intense, cost, and the disposal of hazardous products. So there is the need to find the matrixes with high salt tolerance to enable direct MALDI MS analysis of protein and peptide samples.

It was reported that organic matrix 2,4,6-trihydroxyacetophenone (2,4,6-THAP) combined with diammonium citrate can not only decrease the production of alkaline cation adduct but also improve the intensity of ion signal of peptides and phosphopeptides.[35,36] We speculate that the presence of carbonyl group in the organic matrix 2,4,6-THAP and ammonium ion in the diammonium citrate may play an important role for the enhanced desorption/ionization of phosphopeptides. Therefore, the matrix candidate 3,4-diaminobenzophenone (DABP) has been chosen to evaluate the performance of desorbing/ionizing peptides and phosphopeptides based on the above hypothesis in MALDI MS analysis.

EXPERIMENTAL SECTION

Materials and Reagents. l-1-(Tosylamid)-2-phenylethyl chloromethylketone (TPCK)-treated trypsin (EC 2.4.21.4, from bovine pancreas), neurotensin (acetate salt, synthetic), adrenocorticotropic hormone fragment 18−39 (hormone), insulin, bovine serum albumin (BSA), chicken egg albumin (99%), CHCA, and DHB were purchased from Sigma Chemical (St. Louis, MO). DABP and guanidine hydrochloride (98%) were obtained from Acros Organics Co. Dithiothreitol (DTT) and iodoacetamide (IAA) were purchased from Sina-American Biotechnology Co. (Beijing, China). Sodium dihydrogen phosphate, disodium hydrogen phosphate, urea, and sodium chloride was obtained from Shenyang Lianbang Reagent Co. (Shenyang, China); ammonium bicarbonate was purchased from Beijing Shiji Hongxing Co. (Beijing, China). All the other chemicals were of high-purity chemical reagent grade. The deionized water used in all procedures was purified with a Mill-Q water system (Millpore, Milford, MA).

Sample Preparation for MS Analysis. DABP matrix was dissolved in methanol/water (80/20, v/v) solution containing 3% HCl at a concentration of ~14 mg/mL. A saturated solution of CHCA in acetonitrile (ACN)/H_2O (33/67, v/v) in 0.1% TFA was freshly prepared each day. DHB was dissolved in ACN/H_2O (33/67, v/v) in 0.1% TFA at 20 mg/mL. BSA (0.8 mg) was dissolved in 1 mL of denaturing buffer solution (8 M urea, 50 mM ammonium bicarbonate, pH 8.2 or 6 M guanidine, 50 mM ammonium bicarbonate, pH 8.2), and chicken egg albumin (0.8 mg) was dissolved in 1 mL of reducing solution (8 M urea, 50 mM ammonium bicarbonate, pH 8.2) for 4 h, each mixed with 20 μL of 50 mM DTT. The mixture was incubated at 37 °C for 2 h, and then 40 μL of 50 mM IAA was added and the resultant mixture incubated for an additional 30 min at room temperature in darkness. After that, the BSA and egg albumin solution was diluted with 50 mM ammonium bicarbonate buffer (pH 8.2) for eight times and incubated overnight at 37 °C with trypsin at an enzyme/substrate ratio of 1:40 to produce a proteolytic digest for MALDI TOF MS analysis. The sample solution and matrix solution were mixed 1:1 in an Eppendorf tube and ~0.5 μL of the mixed solution was deposited onto a MALDI target plate.

MALDI-TOF MS Analysis. MALDI-TOF MS measurements were performed on a Bruker Autoflex time-of-flight mass spectrometer (Bruker Co., Bremen, Germany), equipped with a pulsed nitrogen laser operated at 337 nm, capable of executing a linear mode. All mass spectra reported were obtained in the linear mode and calibrated using an external calibration equation generated from the ion signal of angiotension II and insulin chain B. Typically, 30 laser shots were added for per spectrum.

RESULTS AND DISCUSSION

A potential matrix candidate should have a strong absorbance at 337 nm at which the sample is irradiated by pulse laser. As shown in Figure 1a, DABP (3.7×10^{-5} M) in the acidified water/methanol (v/v, 20/80) solution containing 3% HCl does have strong absorbance at 337 nm and the absorption coefficient was determined to be 7660 cm^{-1} M^{-1}. The mass spectrum for direct MALDI analysis of the acidified DABP in the positive ion mode is shown in Figure 1b. It can be seen that this compound was ionized as $[M + H]^+$, $[2M + H]^+$, and $[2M + H - H_2O]^+$ under laser irradiation. The results imply that the compound DABP can be used as a MALDI matrix. The structure of the acidified DABP by hydrogen chloride is as follows:

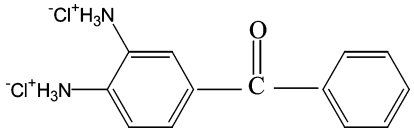

Figure 2 presents the mass spectra of adrenocorticotropic hormone fragment 18−39 (hormone) (m/z 2466, 45 fmol) and neurotensin (m/z 1673, 41 fmol) obtained using matrixes of DABP, CHCA, and DHB, respectively. Similar ion signal pattern and resolution for the analysis of hormone and neurotensin were obtained by the three matrixes, but the ion signal is weak when using DHB as a matrix. This is probably because the same strength of laser power was applied under investigation, and the threshold value of DHB is the highest among those matrixes. Thus, the laser power is too low for MALDI analysis of the peptide sample in our study. Due to the fact that the matrix CHCA is a "hot" matrix, fragmentation of analytes such as peptides is easily

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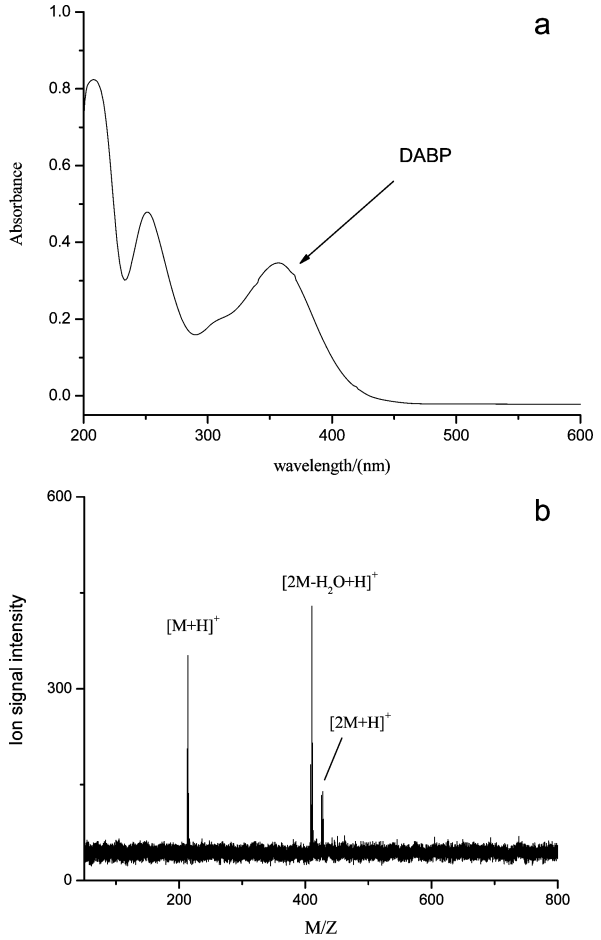

Figure 1. (a) UV spectrum of compound 3, 4-DABP (3.7×10^{-5} M) dissolved in methanol/H_2O (v/v, 80/20) containing 3% HCl; (b) the direct laser desorption/ionization mass spectrum of the acidified compound 3, 4-diamino- benzophenone in the positive ion mode.

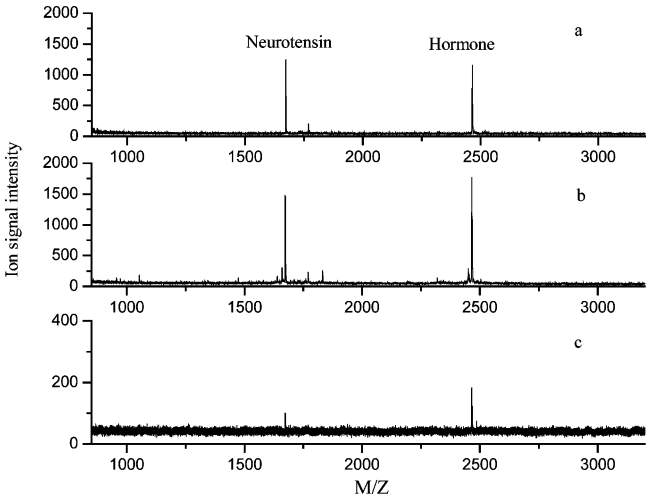

Figure 2. MALDI mass spectra of a mixture of two peptides of neurotensin (41 fmol) at *m*/*z* 1673 and hormone (45 fmol) at *m*/*z* 2466 using (a) DABP, (b) CHCA, (c) DHB as matrixes, respectively.

produced in MALDI MS analysis. As shown in Figure 2b, the mass spectrum of hormone and neurotensin using CHCA as matrix become more complex than that obtained using DABP and DHB as matrixes.

An important aspect for the application of MALDI matrix DABP is the detection sensitivity for analyzing the samples, which was evaluated for the analysis of hormone and neurotensin using matrixes DABP, DHB, and CHCA. It was observed that about 4 fmol of hormone and 5 fmol of neurotensin could be detected by using matrixes of DABP and CCA. The detection limit of hormone and neurotensin with matrix DABP was estimated to be about 3 and 4 fmol, respectively.

Although a conventional matrix such as DHB and CHCA has been successfully applied to the detection of various compounds, the ability of desorption/ionization of these organic matrixes for the analytes will be greatly decreased in the presence of residual amounts of contaminants such as urea and guanidine hydrochloride in MALDI MS analysis.[32,33] It was found that the new matrix, DABP, can tolerate high concentrations of denaturants such as urea and guanidine hydrochloride in sample solution in MALDI MS analysis, especially the presence of guanidine hydrochloride. Figure 3a and b presents the MALDI mass spectra of insulin (400 fmol) in 4 and 2 M guanidine hydrochloride solutions using DABP as matrix. Although the intensity of the ion signal was decreased at higher guanidine hydrochloride concentrations, the detection of insulin could be achieved even in the presence of 4 M guanidine hydrochloride. However, no ion signal of insulin (400 fmol) was observed in the presence of only 1 M guanidine hydrochloride when DHB and CHCA were used as matrixes. Figure 3c presents the MALDI mass spectrum of insulin (400 fmol) in 1.5 M urea solution using DABP as matrix. As we can see, a decent ion signal of insulin was detected. Similarly, no ion signal of insulin (400 fmol) in 1.5 M urea can be observed using CHCA and DHB as matrixes. When the concentration of urea is increased to 2 M, the formation of crystals became very difficult, because the deposited sample mixture of DABP and urea on the target does not dry due to the absorption of water in the atmosphere. The tolerance limit of matrix DABP for urea was determined to be ~1.8 M. The detection limit of insulin using matrix DABP in the presence of 1 M urea and guanidine hydrochloride was also determined. Figure 3d shows MALDI mass spectrum of insulin (40 fmol) using DABP as matrix in the presence of 1 M guanidine hydrochloride. Obviously, the ion signal of insulin (40 fmol) still remains detectable in mass spectrometric analysis. However, it is difficult to obtain the ion signal when the amount of insulin was decreased to 20 fmol in the presence of 1 M guanidine hydrochloride. For the analysis of insulin sample solutions in the presence of 1 M urea, a similar result as for the sample solution in the presence of guanidine hydrochloride was obtained.

To test the applicability of the new matrix to a more complex sample, the tryptic digest of BSA was analyzed with a matrix of DABP. Before the protein is digested with a site-specific proteolytic enzyme, a denaturant such as urea or guanidine hydrochloride is often used for the denaturalization of protein. Matrix DABP was directly used to analyze the tryptic digest of protein BSA in 1 M guanidine hydrochloride and 1 M urea solutions without any pretreatment prior to MALDI MS analysis. The MALDI mass spectrum of the tryptic digest of protein BSA (400 fmol) with 1 M guanidine hydrochloride is presented in Figure 5a. As can be seen, it is obvious that the mass information from peptide residues can be obtained in the mass spectrum. After database searching, sequence coverage of BSA obtained using DABP as the matrix has reached 78.0%. However, no ion signals of peptide residues

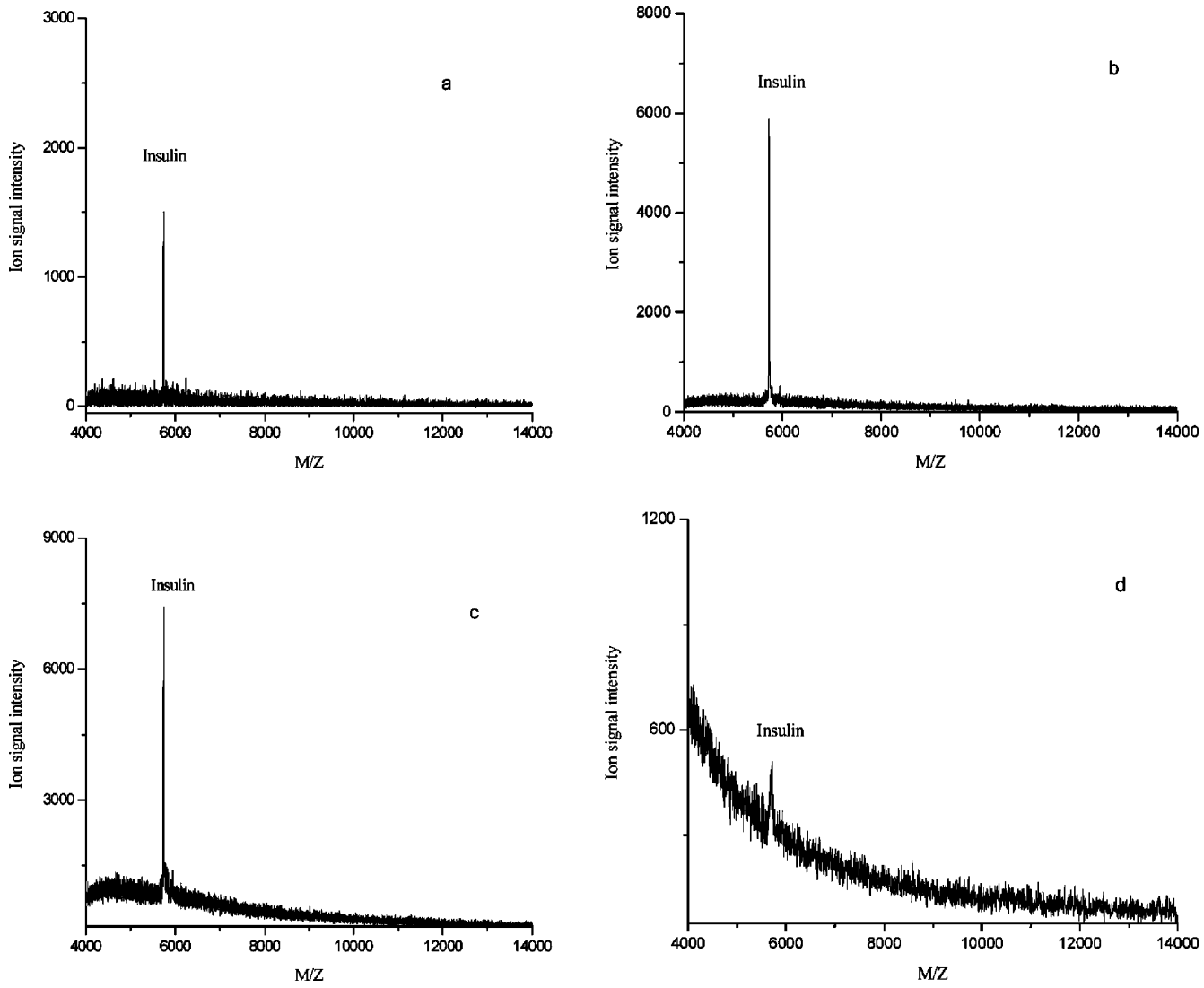

Figure 3. MALDI mass spectra of analyte insulin (400 fmol) using DABP as matrix in the presence of contaminants: (a) 4 M guanidine hydrochloride, (b) 2 M guanidine hydrochloride, (c) 1.5 M urea, and (d) 40 fmol of analyte insulin in 1 M guanidine hydrochloride.

were detected when DHB and CHCA were used as matrixes. Then the tryptic digest of protein BSA (400 fmol) containing 0.1 M guanidine hydrochloride was analyzed by using matrixes DABP, DHB, and CHCA, respectively. The corresponding sequence coverage of BSA (400 fmol) obtained is 79.2, 57.1, and 72%, respectively. Figure 5b shows the MALDI mass spectrum of the tryptic digest of protein BSA (400 fmol) in 1 M urea solution using DABP as matrix. Sequence coverage of 66.6% was obtained. Similarly, no ion signal of peptide residues of BSA was observed using DHB and CHCA as matrixes. Furthermore, the tryptic digest of protein BSA (400 fmol) in 0.1 M urea solution was also tested by using matrixes DABP, DHB, and CHCA, respectively. And the obtained sequence coverage is 77.7, 59.0, and 67.4%. All of the above results indicated that the presence of 1 M guanidine hydrochloride and 1 M urea in sample solution does not seriously interfere with the detection of a tryptic digest of BSA using DABP as the matrix in MALDI MS analysis. Particularly with the presence of 1 M guanidine hydrochloride, there is almost no effect for the detection of thr tryptic digest of BSA (400 fmol) compared to the presence of 0.1 M guanidine hydrochloride according to the obtained sequence coverage. However, it is impossible for the detection of the tryptic digest of protein BSA using conventional matrixes DHB and CHCA. Considering the diversity of peptides present in a BSA digest and the good sequence coverage obtained, this new matrix should also be able to analyze other peptides with a high concentration of denaturants. Peptide mapping analysis with MALDI-TOF MS is a frequently used technique for identification of proteins and posttranslational modification.[35] Purification of protein digest prior to MALDI analysis is typically required for a conventional matrix. Our new matrix can simplify the procedure of peptide mapping analysis of protein in MALDI analysis.

Figure 4a shows the MALDI mass spectrum of cytochrome *c* (300 fmol), myoglobin (300 fmol), and BSA (400 fmol) in the presence of 1 M guanidine hydrochloride using DABP as matrix. Obviously, the ion signal of analytes cytochrome *c*, myoglobin, and BSA is detected in the mass spectrum. The MALDI mass spectrum of cytochrome *c* (300 fmol), myoglobin (300 fmol), and BSA (400 fmol) in 1 M urea is shown in Figure 4b using DABP as the matrix. The ion signal of cytochrome *c* (300 fmol) and myoglobin (300 fmol) obtained is strong, but the ion signal of BSA is weak in comparison to the ion signal of of cytochrome *c*

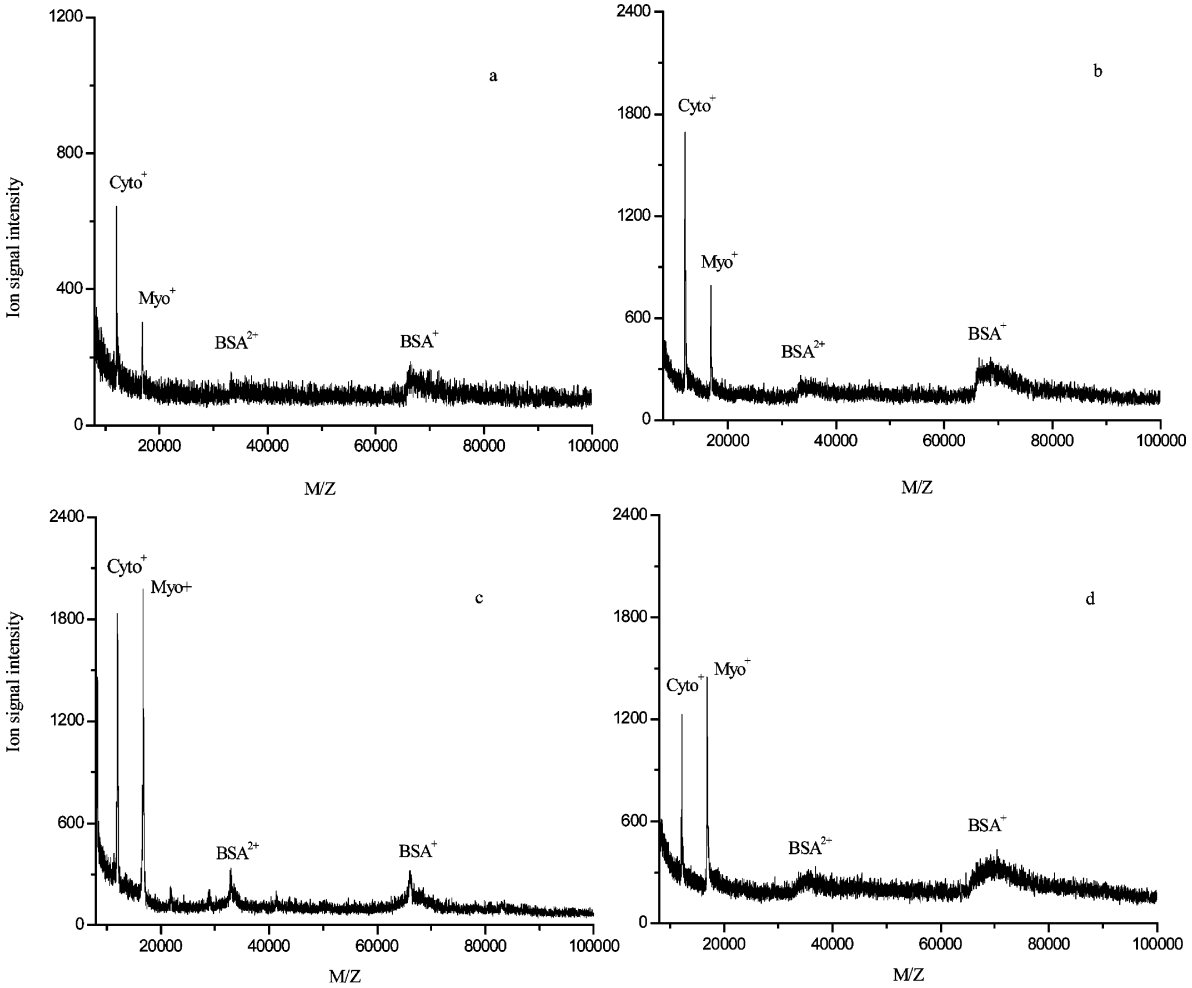

Figure 4. MALDI mass spectra of analytes cytochrome *c* (300 fmol), myoglobin (300 fmol), and BSA (400 fmol) in the presence of (a) 1 M guanidine hydrochloride and (b) 1 M urea using DABP, and in the absence of denaturants using matrixes of (c) CHCA and (d) DABP.

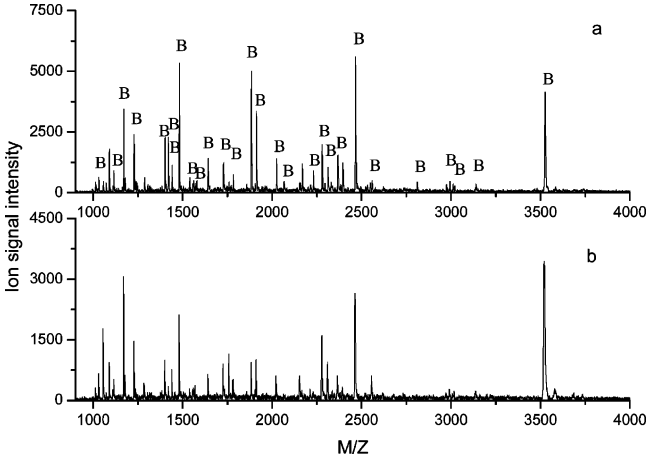

Figure 5. MALDI mass spectrum of the tryptic digest of protein BSA (400 fmol) in the presence of (a) 1 M guanidine hydrochloride and (b) 1 M urea using DABP as matrix. B stands for peptide digested from protein BSA.

and myoglobin. Subsequently, we further compared the ion signal of analytes (BSA) without any denaturant in the sample solution when using DHB, CHCA, and DABP as matrixes. Figure 4c displays the MALDI mass spectrum of cytochrome *c* (300 fmol), myoglobin (300 fmol), and BSA (400 fmol) when using CHCA as the matrix, and a similar result was obtained using DHB as the matrix. It can be seen that the ion signal of BSA in the mass spectrum is better than that obtained using DABP as the matrix (Figure 4d). It seems that the new matrix DABP only works well for peptides and small proteins. To investigate the performance of this matrix thoroughly, a detailed study of more protein samples will be performed in the later work.

Alkali metal cation adduction such as Na^+ and K^+ for the analysis of peptides is a pervasive phenomenon using CHCA and DHB as matrixes in the MALDI MS analysis. It has been found that matrix DABP can effectively suppress the formation of the cation ion adducts of analyte in the MALDI MS analysis. The peptides of neurotensin (80 fmol) and hormone (70 fmol) in the 50 mM NaH_2PO_4−Na_2HPO_4 buffer solution (pH 7.0) were selected to evaluate the capability of matrix DABP to sequester monovalent cations such as Na^+ and K^+. The MALDI mass spectra of neurotensin and hormone in the presence of 50 mM NaH_2PO_4−Na_2HPO_4 buffer solution are shown in the Figure 6a and b using CHCA and DHB as matrixes, respectively, and the peptides with adducts $[M + Na - H]^+$ and $[M + 2Na - 2H]^+$ are the most prevalent ion signals except for $[M + H]^+$. However, almost no ion signal of neurotensin and hormone with metal ion adduct in 50 mM NaH_2PO_4−Na_2HPO_4 buffer solution was detected in the MALDI MS using DABP as matrix (Figure 6c). The presence of

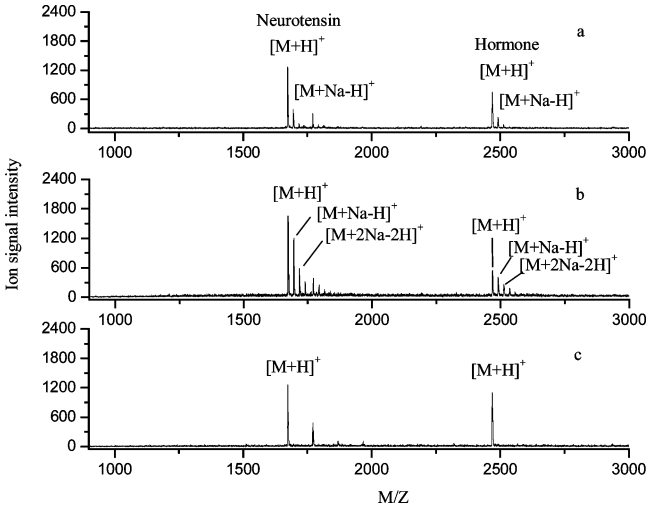

Figure 6. MALDI mass spectra of peptides neurotensin (80 fmol) and hormone (70 fmol) in 50 mM $NaH_2PO_4-Na_2HPO_4$ buffer solution (pH 7.0) using (a) CHCA, (b) DHB, and (c) DABP as matrixes.

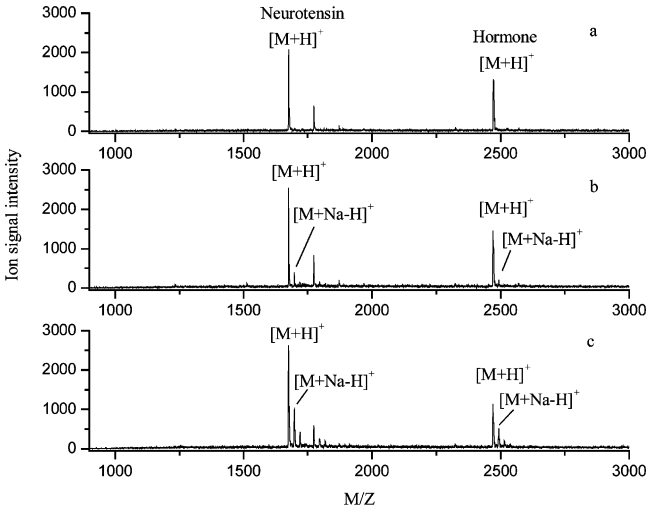

Figure 7. MALDI mass spectra of peptides neurotensin (80 fmol) and hormone (70 fmol) using DABP as matrix in solutions containing (a) 50 mM, (b) 200 mM, and (c) 2.5 M sodium chloride, respectively.

sodium ion adduct peaks complicated the mass spectrum and decreased the detection sensitivity of peptides.

To further evaluate the limit of this matrix DABP for the metal ion adduct, the peptides neurotensin (80 fmol) and hormone (70 fmol) in 50, 100, 200, and 500 mM and 2.5 M NaCl solutions were used to test cation ion adductions. Figure 7 shows the MALDI mass spectrum of peptides neurotensin and hormone in the presence of (a) 50 mM, (b) 200 mM, and (c) 2.5 M NaCl. It can be seen that almost no metal ion adduction of peptide neurotensin and hormone can be observed in the presence of 50 mM NaCl in the MALDI MS analysis, as shown in Figure 7a. When the concentration of NaCl in the sample solution reached 200 mM, sodium cation adduction was also observed in the mass spectrum using DABP as matrix (Figure 7b). In the presence of 2.5 M NaCl, the ion signal of the neurotensin (80 fmol) and hormone (70 fmol) is also detected, but the sodium adduction peaks dominate the whole mass spectrum (Figure 7c).

The mechanism of depressing the metal ion adductions using DABP as matrix is of interest in MALDI mass spectrum analysis. The mechanism may be similar to that of using tetraamine and ammonium ions as additives to the organic matrix to suppress the formation of metal ion adductions.[38−40] The two amino groups of the compound DABP are converted to ammonium ions after the addition of 3% HCl. When samples containing sodium contaminants are mixed with matrix DABP, the sodium salt probably precipitates first during the cocrystallization procedure, thereby decreasing the sodium ion concentration on the surface of cocrystallized matrix with sample molecules. In addition, there is a strong hydrophobic group on the matrix DABP, which may have a hydrophobic interaction with the sample molecules. On the other hand, the presence of ammonium cation on matrix DABP hinders the contact of sodium ion with the sample molecules. So the metal ion adductions for sample molecules can effectively be suppressed in the MALDI analysis. If the concentration of sodium salt preponderates over the tolerance limit of matrix DABP for the cation ion adductions, the sodium salt also accumulates on the surface of formed crystal with analyte and matrix on the metal target; therefore, the cation ion adductions cannot be eliminated, which can be supported from the results shown in Figure 7c. Furthermore, when peptides were analyzed using the unacidified DABP as matrix, although the ion signal of analytes is very strong, the sodium ion adductions of analytes were not suppressed. It is demonstrated that the formation of ammonium ion on matrix DABP molecule through an acidifying process plays an important role in the desalting procedure. Although the combination of other organic matrixes such as THAP with some amount of ammonium salt can suppress the cation ion adduction of analytes to some degree, the peptide signals were dramatically decreased with the addition of ammonium citrate at concentrations of 5 mM or higher,[41] and this continues to be an empirical consideration.

Phosphorylation, the most important and ubiquitous posttranslational modification of proteins, is able to regulate almost all aspects of cell life in both prokaryotes and eukaryotes.[42−44] The determination of phosphorylation sites in these phosphorylated proteins is an important step in the understanding of the molecular basis of these processes. The preferred approach for the identification of phosphorylation sites of proteins mainly relies on the use of mass spectrometry to sequence individual peptides after proteolysis. However, phosphopeptides are often of low ion signal intensity compared with that from unmodified peptides, presumably owing to their low ionization efficiency in the positive ion mode. A tryptic digest of chicken egg albumin was used for the characterization of DABP, DHB, and CHCA as matrixes in MALDI MS analysis. The positive ion MALDI-TOF mass spectra of a tryptic digest of chicken egg albumin (100 fmol) in 100 mM urea solution obtained using matrixes DABP, DHB, and CHCA are presented in Figure 8a−c, respectively. The deprotonated phosphopeptides at m/z 2091 and 2903 were detected using either

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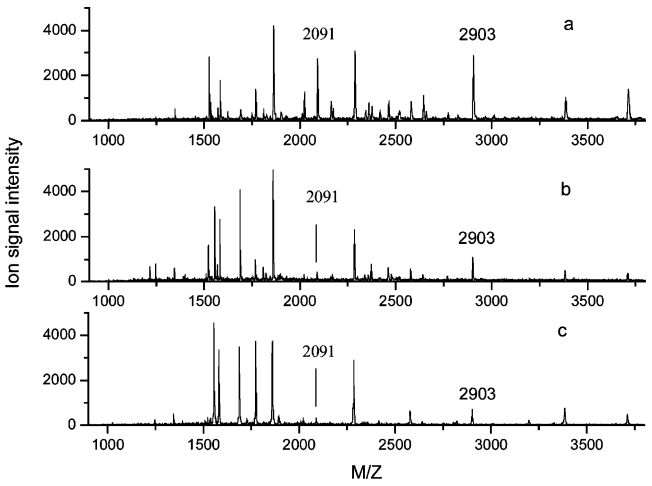

Figure 8. MALDI mass spectra of the tryptic digest of protein chicken egg albumin (100 fmol) in 100 mM urea using (a) DABP, (b) DHB, and (c) CHCA as matrixes in the positive ion mode.

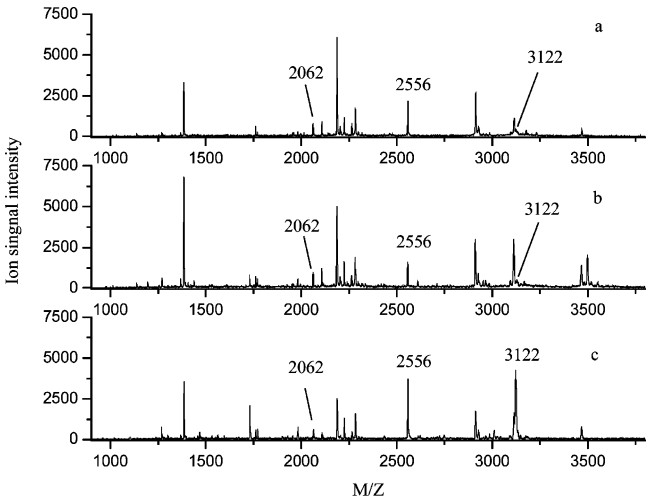

Figure 9. MALDI mass spectra of the tryptic digest of β-casein (200 fmol) using (a) CHCA, (b) DHB, and (c) DABP as matrixes in the positive ion mode.

matrix. When the DHB and CHCA were used as matrixes to detect the tryptic digest of chicken egg albumin, the intensity of ion signal of phosphopeptides at m/z 2091 and 2903 was rather low as shown in Figure 8b and c. However, the intensity of the ion signal of those two phosphopeptides is enhanced using DABP as the MALDI matrix. It is indicated that the DABP matrix can enhance the detection of phosphopeptides in a tryptic digest of chicken egg albumin in MALDI MS analysis. We next investigated whether the detection of phosphopeptides could be further improved by switching to the negative ion mode in MALDI MS analysis. Similar to the positive ion mode, the phosphopeptides at m/z 2089 and 2901 were observed using DABP, DHB, and CHCA as matrixes in negative ion mode of the MALDI MS analysis. The obtained results showed that the intensity of ion signal of phosphopeptides from the digest of chicken egg albumin was increased to some degree in either positive or negative mode using matrix DABP rather than DHB and CHCA. The tryptic digest of chicken egg albumin has been reported to contain three single phosphorylated peptides at m/z, 2091, 2513, and 2903, respectively.[45,46] However, one monophosphopeptide at m/z 2513 was not observed either in the positive mode or in the negative mode using DABP, DHB, and CHCA as matrixes in our study.

A peptide mixture generated by tryptic digestion of β-casein was also investigated using matrixes of DABP, DHB, and CHCA in MALDI MS analysis. The tryptic digest of phosphoprotein β-casein contains three phosphopeptides at m/z 2062, 2556 and 3122.[45] Figure 9 shows MALDI mass spectra of the tryptic digest of β-casein (200 fmol) using matrixes of CHCA, DHB, and DABP, respectively. It is obvious that two monophosphopeptides at m/z 2062 and 2556 can be detected using each of the three matrixes, but the ion signal of the phosphopeptide containing four phosphoric acid groups is very weak in MALDI MS analysis with matrixes DHB and CHCA as shown in Figure 9a and b. However, when using DABP as the matrix to detect the tryptic digestion, the ion signal of the multiple phosphopeptide at m/z 3122 has been greatly enhanced in the mass spectrum as shown in Figure 9c.

CONCLUSION

It has been demonstrated that DABP is a good matrix candidate for the analysis of a mixture of peptides in high concentrations of contaminants such as urea and guanidine hydrochloride by MALDI-TOF MS. In the presence of 4 M guanidine hydrochloride, the ion signal of insulin (400 fmol) was detected with the MALDI mass spectrum, and the detection limit of insulin approached 40 fmol with the presence of 1 M guanidine hydrochloride. Due to the difficulty in forming a crystal at high urea concentration, matrix DABP for the analysis of insulin (400 fmol) can tolerate ~1.8 M urea. Furthermore, almost no change of the sequence coverage of protein BSA (400 fmol) in the presence of 1 and 0.1 M guanidine hydrochloride was observed in MALDI MS analysis. It was also observed that matrix DABP can effectively suppress the cation ion adduction in the presence of a high concentration of metal ion for analysis of peptides in MALDI-TOF MS. However, the metal ion adductions can also form in the presence of 200 mM NaCl for the peptides neurotensin (80 fmol) and hormone (70 fmol). When the concentration of NaCl is increased to 2.5 M, the metal ion adductions also dominate the whole mass spectrum. Finally, the ion signal of phosphorylated peptides in a tryptic digest of chicken egg albumin and β-casein was enhanced to some degree using matrix DABP rather than DHB and CHCA in the positive and negative ion MALDI MS analysis, especially for the multiple phosphopeptides.

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