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Iminodiacetic acid derivatized porous silicon as a matrix support for sample pretreatment and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis

Songyun Xu, Houjiang Zhou, Chensong Pan, Yu Fu, Yu Zhang, Xin Li, Mingliang Ye and Hanfa Zou*

National Chromatographic R & A Center, Dalian Institute of Chemical Physics, The Graduate School of Chinese Academy of Sciences, Chinese Academy of Sciences, Dalian 116011, China

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Iminodiacetic acid (IDA)-1,2-epoxy-9-decene has been synthesized and covalently linked to the surface of porous silicon wafer through a photochemical reaction. The negatively charged carboxylic acid groups on the porous silicon wafer are capable of binding oppositely charged species from sample solutions through electrostatic interactions. This allows the removal of contaminants prior to matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) by simply washing the porous silicon surface. The carboxylic acid end groups on porous silicon can be used to selectively bind and concentrate target species in sample solutions. Furthermore, Fe^{3+}-IDA-derivatized porous silicon was prepared to specifically and effectively concentrate phosphopeptides from the tryptic digests of phosphoproteins, followed by MALDI-MS analysis. Copyright © 2006 John Wiley & Sons, Ltd.

The advent of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS)[1] resulted in the rapid development of new bioanalytical methods, which allow characterization of the species of interest in a rapid, sensitive, and accurate way. Mixtures can be directly analyzed by the MALDI-TOFMS technique without tedious pretreatment. However, there are still some unresolved challenges in the general application of MALDI-TOFMS. For example, contaminants, such as salts or surfactants, in solution will strongly suppress ionization of analyte molecules in MALDI-TOFMS, because these contaminants probably disrupt the co-crystallization of analytes and matrix molecules.[2] Some novel techniques such as surface-enhanced desorption/ionization (SELDI) have been developed to circumvent these problems.[2–24] Usually, salts and contaminants in sample solution can be selectively removed by these methods. For example, if a sample solution containing the contaminants is deposited onto a hydrophobic surface such as polyethylene (PE),[18] polypropylene,[13] paraffin,[12] or Teflon,[16] the hydrophilic contaminants can be removed by washing the surface. A PE-modified MALDI probe was used as a sample support to obtain a mass spectrum of bovine serum albumin in a solution containing 0.73% sodium dodecyl sulfate (SDS), after washing the sample probe.[18] Diamond nanoparticles and gold nanoparticles have also been employed to selectively capture target species from highly diluted and contaminated sample solutions.[7,17]

Porous silicon obtained from flat crystalline silicon by a simple galvanostatic etching procedure has attracted great attention for its extraordinary material properties such as its high surface area-to-volume ratio (hundred of square meters per cubic centimeter), pore geometry, morphology, and the optical properties of the porous layer.[25,26] Freshly etched porous silicon surfaces are hydrophobic owing to the presence of the metastable, silicon-hydride terminal groups, but the surface of porous silicon can be easily stabilized and functionalized as required through Lewis-acid-mediated[27] or light-promoted hydrosilylation reactions.[28] Porous silicon has been used for the analysis of biomolecules as an immobilized matrix in micro-enzyme reactors,[29–33] and as a protein chip substrate for the immobilization of antibodies.[26] A matrix-free, desorption/ionization on porous silicon (DIOS)-MS technique was also developed by Siuzdak and co-workers.[34,35] In this method, porous silicon was used as the matrix for the desorption/ionization of analytes in MALDI, to minimize fragmentation of the analyte and to eliminate interference from matrix ions in the low mass range.

In this study, iminodiacetic acid (IDA)-1,2-epoxy-9-decene with two negatively carboxylic acid end groups has been

*Correspondence to: H. Zou, National Chromatographic R&A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116011, China.
E-mail: hanfazou@dicp.ac.cn
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synthesized and covalently bound to the surface of porous silicon wafer through a photochemical reaction. Positively charged analytes in sample solutions can be trapped on the IDA-derivatized porous silicon, and then analyzed by MALDI-TOFMS. Proteins, cationic surfactants, and egg white were used to evaluate the performance of the IDA-derivatized porous silicon. Similarly, the prepared Fe^{3+}-IDA-derivatized porous silicon wafer can be applied to specifically and effectively concentrate phosphopeptides from the tryptic digests of phosphoproteins prior to MALDI-TOFMS analysis.

EXPERIMENTAL

Materials

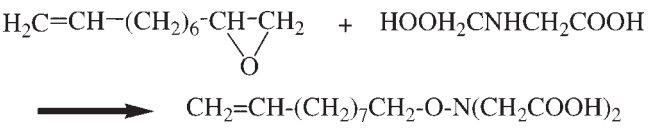
Horse heart cytochrome C, 1-1-(tosylamide)-2-phenylethyl-chloromethyl ketone (TPCK)-treated trypsin (EC 2.4.21.4, from bovine pancreas), myoglobin (from horse skeletal muscle) and α-cyano-4-hydroxycinnamic acid (CHCA) were purchased from Sigma Chemicals (St. Louis, MO, USA). Lysozyme (Serva, 28262 4C, from egg white) was obtained from Sina-American Biotechnology Co. (Beijing, China). Tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and decyltrimethylammonium bromide were obtained from Fluka (Buchs, Switzerland). 1,2-Epoxy-9-decene and iminodiacetic acid (IDA) were obtained from Aldrich (St. Louis, MO, USA). Phosphorus-doped, n-type silicon single crystals (100), with resistivity of $1–2 \times 10^{-2}\ \Omega \cdot$cm, were supplied by the Beijing Institute of Non-Metals (Beijing, China). Fresh eggs were purchased from a local supermarket (Dalian, China). All other chemicals were of high purity chemical reagent grade. The deionized water used in all procedures was purified with a Mill-Q water system (Millipore, Milford, MA, USA). Usually, proteins were dissolved in 50 mM phosphate-buffered solution (PBS) at pH 7.0.

Preparation of porous silicon support

A low-resistivity silicon wafer was tailored to 1 cm × 1 cm, and then it electrochemically etched in a solution of ethanol/49% HF (2:3, v/v) for 2 min in a custom-built Teflon cell under the illumination of a 250-W tungsten filament bulb with a Pt wire as counter electrode. The current density was 8 mA/cm^2. The product from the above procedure was further processed by rapid oxidation with 30% ozone for 1 min and re-etched with 5% (v/v) HF in ethanol for 1 min. After washing with ethanol, the prepared porous silicon wafers were stored in oxygen-free ethanol until required.

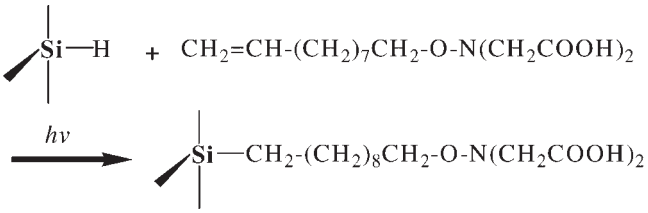
Synthesis of IDA-1, 2-epoxy-9-decene

IDA (1.31 g, 0.01 mol) was dissolved in 30 mL of 50 mM Na_2CO_3 solution, and the solution adjusted to pH 10.5 with 2 M NaOH. The solution was then transferred to a 100-mL round-bottomed flask in an oil bath. The mixture was gradually heated to 65°C, and 1,2-epoxy-9-decene (0.5 mL, 3.5 μmol) was slowly added from a dropping funnel at a rate of one drop per 2 min. The reaction proceeded for 20 h while being stirred with a magnetic stir bar. Similarly, another 0.5 mL of 1, 2-epoxy-9-decene was added to the reaction mixture at the same rate from the dropping funnel over 20 h. Finally, the solution of the synthesized IDA-1, 2-epoxy-9-decene was adjusted to pH 7.0 with 0.2 M HCl containing 50% ethanol and stored in the refrigerator until required. The procedure for the synthesis of IDA-1, 2-epoxy-9-decene is shown in the following scheme:



Immobilization of IDA-1, 2-epoxy-9-decene on the porous silicon

Hydrosilylation of porous silicon was carried out in a glass flow cell containing double layers with inlet and outlet ports for the circulation of cooling water. IDA-1,2-epoxy-9-decene solution (6 mL) was added to the flow cell, where the porous silicon was immersed in the solution. Butyl acetate (2 mL) was then added to cover the surface of the IDA-1,2-epoxy-9-decene solution, to decrease the evaporation of the sample solution. Light from a 1000-W Hg bulb was focused on the porous silicon at a distance of approximately 15 cm from the surface. After illumination for 2 h, the porous silicon was removed from the flow cell, rinsed with ethanol and dried under a flow of nitrogen. The procedure for the immobilization of IDA-1, 2-epoxy-9-decene on the porous silicon surface is shown in the following scheme:



Sample pretreatments

The CHCA matrix was dissolved in a solution of acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA) (40:60, v/v) at a concentration of 7 mg/mL. A mixture of tetradecyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and hexadecyltrimethylammonium bromide in 100 mM PBS (pH 7.0) at 0.1 mg/mL was selected to examine the desorption/ionization of analytes on the IDA-bound porous silicon. The mixture of three surfactants (1 μL) was directly spotted onto the IDA-bound porous silicon and incubated for 5 min. The porous silicon was vortexed for 30 s in 100 mM phosphate buffer containing 200 mM NaCl, and then washed with deionized water. The porous silicon was then dried and the trapped surfactants on the surface were analyzed directly by DIOS-MS.

Egg white, obtained from a fresh egg, was dissolved in 100 mM phosphate buffer at 1:19 dilution. The diluted egg white sample (20 μL) was loaded on the surface of the IDA-derivatized porous silicon with equilibrium buffer at different pH values and incubated for 5 min. The IDA-derivatized porous silicon was then vortexed in 100 mM phosphate buffer containing 200 mM NaCl for 30 s and further washed by deionized water. Then, 6 μL of the solution of the CHCA matrix were added to the surface of the

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porous silicon to form co-crystals with the trapped sample molecules for MALDI analysis.

Protein sample solutions with pH lower than 10, or at 12.2, were prepared in 100 mM phosphate buffer or 100 mM Na_2CO_3 buffer, respectively. β-Casein (10^{-6} M) was digested by trypsin in a 50 mM NH_4HCO_3 solution for 16 h at 38°C with a protein-to-trypsin ratio of 40:1 (w/w).

Immobilization of Fe^{3+} on the IDA-derivatized porous silicon

The surface of the IDA-derivatized porous silicon was first washed with 1 mL 0.1% acetic acid (HAc) solution, flushed with 50 mM EDTA solution, and then washed with 2 mL 0.1% HAc solution to remove residual EDTA on the surface. The IDA-derivatized porous silicon was incubated with 100 mM $FeCl_3$ solution for 30 min at 20°C. The Fe^{3+}-IDA-derivatized porous silicon obtained was then washed with 2 mL 0.1% HAc solution to remove any weakly bound metal ions.

The protocol of phosphopeptide enrichment

The Fe^{3+}-IDA-derivatized porous silicon was first washed with 5 μL of 100 mM NaCl solution to eliminate the effect of electrostatic adsorption of the porous silicon surface. The tryptic digest of phosphoprotein was dissolved in a solution containing 30% ACN and 100 mM NaCl buffer, and its pH value adjusted to 3–4 with 2% HAc. About 1 μL of the above sample solution was directly loaded onto the Fe^{3+}-IDA-derivatized porous silicon and incubated for 15 min. The Fe^{3+}-IDA-derivatized porous silicon was washed with 2 mL of a solution containing 50% ACN and 100 mM NaCl in 0.1% TFA buffer for 5 min, and then with deionized water. Finally, 2 μL of a 2,5-dihydroxybenzoic acid (DHB) matrix solution was deposited onto the Fe^{3+}-IDA-derivatized porous silicon to form co-crystals with the trapped phosphopeptides from the complex sample solution. The Fe^{3+}-IDA-derivatized porous silicon was directly attached to the MALDI target plate with conductive gel for MS analysis.

Mass spectrometric analysis

All DIOS- and MALDI-MS experiments were performed on a Bruker Autoflex time-of-flight mass spectrometer (Bruker, Bremen, Germany), equipped with a delayed ion extraction device and a pulsed nitrogen laser operated at 337 nm. All mass spectra reported were obtained in the positive ion mode and calibrated using an external calibration equation generated from the spectra of angiotension II and insulin B. Typically, 30 laser shots were added per spectrum.

RESULTS AND DISCUSSION

Immobilization of IDA-1,2-epoxy-9-decene on hydrogen-terminated porous silicon

Stewart and Buriak[28] and James *et al.*[36] have reported covalently bonding alkyl monolayers onto the surface of porous silicon using a photochemical reaction. We applied this approach for the immobilization of 1,2-epoxy-9-decene on the surface of porous silicon in our initial experiment. However, when the 1, 2-epoxy-terminated porous silicon was further reacted with IDA under basic conditions (pH 10.5) at 65°C for 16 h, we found that the porous silicon wafer was eroded to some degree. Therefore, another route was adopted for the immobilization of IDA on the porous silicon. First, IDA was reacted with 1,2-epoxy-9-decene, and the obtained product was immobilized on the hydrogen-terminated porous silicon through a photochemical reaction. Figure 1 shows the MALDI mass spectrum of the product using carbon nanotubes as a matrix,[37] verifying the formation of IDA-1, 2-epoxy-9-decene. In the presence of excess IDA, most of the 1,2-epoxy-9-decene was converted into IDA-1,2-epoxy-9-decene. DIOS-MS with freshly etched porous silicon was also used to analyze the IDA-1,2-epoxy-9-decene, but no spectrum was obtained. This may because the spectrum of IDA-1,2-epoxy-9-decene in DIOS-MS was suppressed by the high concentration of Na_2CO_3 buffer salt in the sample solution.

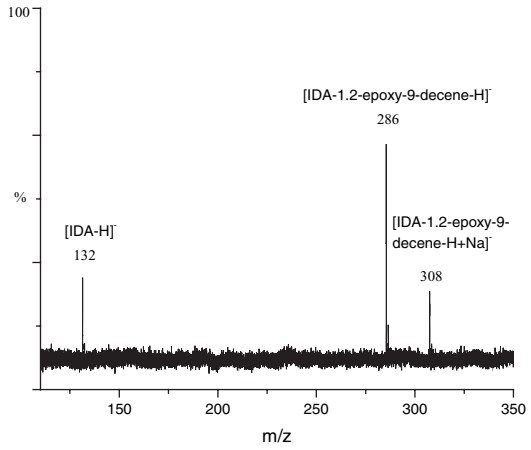

Figure 1. Negative ion MALDI mass spectrum of IDA-1, 2-epoxy-9-decene, using carbon nanotubes as matrix.

Sample pretreatments on IDA-derivatized porous silicon for MALDI-MS analysis

The IDA-derivatized porous silicon, with two negatively charged carboxylic acid end groups extended to the sample solution by a carbon linker, was expected to trap positively charged species from the sample solution. Cationic species usually bind with anions through electrostatic interaction. However, the interaction of cationic surfactants with anions is also affected by hydrophobic interaction at solid-liquid interfaces,[17,38] which increases with the length of the alkyl chain of the cationic surfactants. Similarly, due to the presence of 1,2-epoxy-9-decene groups on the IDA-derivatized porous silicon, the interaction between the analyte and the IDA-terminated porous silicon may depend not only on the charge, but also on the structure and polarity of the analyte. In order to examine the binding interactions between IDA-terminated porous silicon and the target species, a sample solution containing the cationic surfactants of hexadecyltrimethylammonium bromide (C16+), tetradecyltrimethylammonium bromide (C14+), and dodecyltrimethylammonium bromide (C12+) at 0.1 mg/mL was deposited onto the surface and directly analyzed by DIOS-MS. The mass spectrum obtained is shown in Fig. 2(A). Almost the same ion signal intensities were observed for the three

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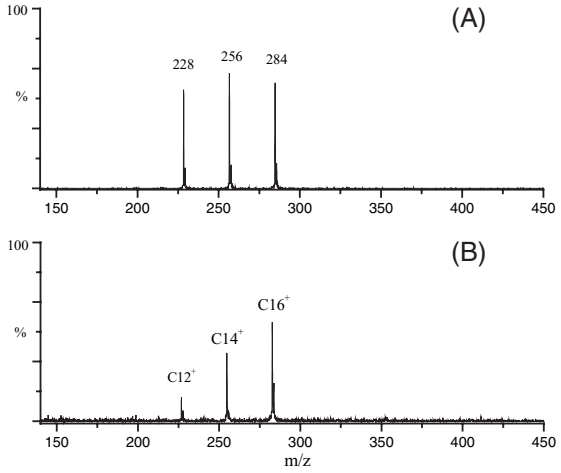

Figure 2. DIOS mass spectra for a mixture of hexadecyltrimethylammonium bromide (C16+), tetradecyltrimethylammonium bromide (C14+), and dodecyltrimethylammonium bromide (C12+) at 0.1 mg/mL. (A) Depositing 1 μL of sample solution on IDA-derivatized porous silicon for direct analysis. (B) Depositing 1 μL of sample solution on IDA-derivatized porous silicon, vortexing for 30 s with 100 mM phosphate buffer containing 200 mM NaCl, and then washing with deionized water.

cationic surfactants, C12+, C14+, C16+ species, at *m/z* 228, 256, and 284, respectively. Furthermore, the same sample solution (1 μL) was also deposited onto the IDA-derivatized porous silicon, and the porous silicon matrix was vortexed in 100 mM phosphate buffer containing 200 mM NaCl and washed with deionized water. The DIOS mass spectrum obtained using this procedure is shown in Fig. 2(B). The spectrum indicates that the cationic surfactant C16+ with the longest alkyl chain length has the highest signal intensity and that the weakest signal was detected for the surfactant C12+ with the shortest alkyl chain. The results reveal that the IDA-derivatized porous silicon surface had different affinities for the cationic surfactants with different alkyl chain lengths. Electrostatic and hydrophobic interactions are involved in the adsorption of cationic target species on the carboxylic acid terminated porous silicon.

It is known that the contaminants such as urea and surfactants in sample solutions can drastically suppress the ion signal of analytes in MALDI-MS.[15,17] Figure 3(A) shows the MALDI mass spectrum of a sample solution (1 μL) of cytochrome C (10^{-5} M) and lysozyme (10^{-5} M) containing 8 M urea deposited onto the IDA-terminated porous silicon surface. It can be seen that almost no signal for the proteins was detected by direct MALDI-MS. However, when the same sample solution (1 μL) was deposited onto the IDA-derivatized porous silicon, and treated by washing and further addition of a CHCA matrix solution (2 μL) to form co-crystals with the trapped species, the mass spectrum (Fig. 3(B)) shows that the proteins can be readily detected. Addition of the CHCA matrix solution to the target surface overcomes the limitation of DIOS-MS in analyzing high molecular weight compounds.[31,39,40] However, the freshly prepared porous silicon is a hydrogen-silicon-terminated

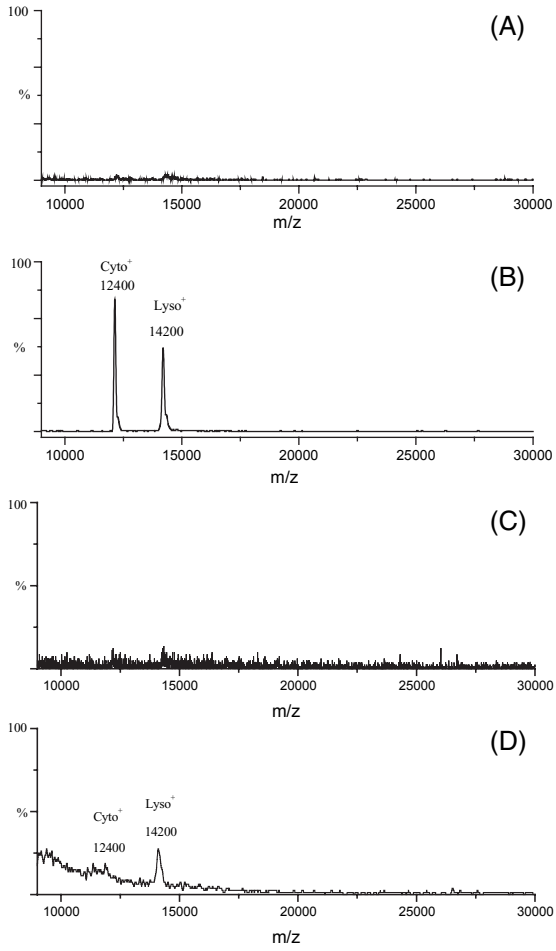

Figure 3. MALDI mass spectra for a mixture of cytochrome C (10^{-5} M) and lysozyme (10^{-5} M) in 8 M urea solution. (A, C) Depositing 1 μL of sample solution on IDA-derivatized porous silicon and hydrogen-terminated porous silicon for direct analysis, respectively. (B, D) Depositing 1 μL of the same sample solution on IDA-derivatized porous silicon and hydrogen-terminated porous silicon with washing procedures to trap target species, respectively. CHCA was used as the MALDI matrix.

surface, having hydrophobic interaction with sample molecules at solid-liquid interfaces. Figure 3(C) presents the MALDI mass spectrum for the analysis of the sample solution (1 μL) of cytochrome C (10^{-5} M) and lysozyme (10^{-5} M) with 8 M urea, directly deposited onto the hydrogen-terminated porous silicon surface. Almost no signal is observed for the proteins in the presence of urea. However, when the hydrogen-terminated porous silicon surface is used to trap the analytes from the same sample solution (1 μL) with 8 M urea followed by washing, weak signals for cytochrome C and lysozyme appear in the mass spectrum (Fig. 3(D)). The mass spectra shown in Figs. 3(B) and 3(D) indicate that the IDA-terminated porous silicon surfaces have a higher affinity than hydrogen-terminated porous silicon surfaces for sample analytes. This is because electrostatic interaction and the hydrophobic interaction of the carbon linker on the IDA-terminated porous silicon surface dominate the adsorption process. However, for the freshly etched porous silicon surface, only the

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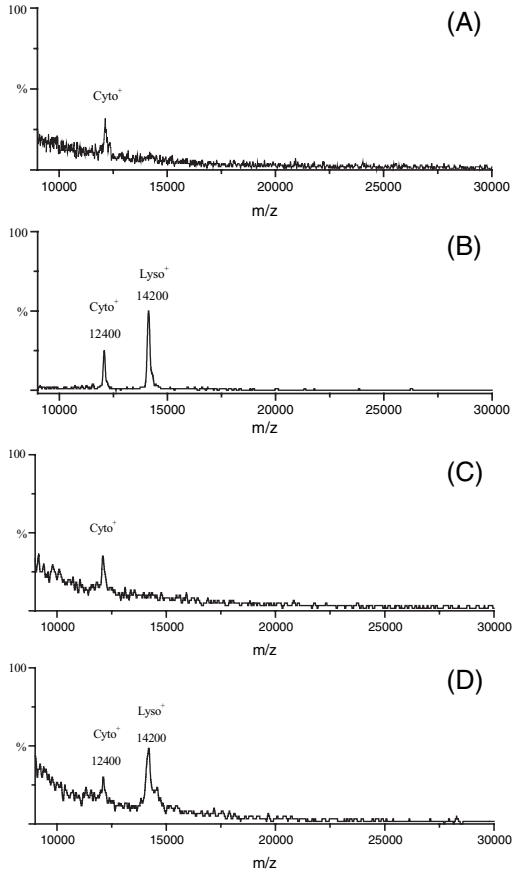

Figure 4. MALDI mass spectra for a mixture of cytochrome C (10^{-5} M) and lysozyme (10^{-5} M) in 10 mM SDS solution. (A, C) Depositing 1 μL of sample solution on IDA-derivatized porous silicon and hydrogen-terminated porous silicon for direct analysis, respectively. (B, D) Depositing 1 μL of the same sample solution on IDA-derivatized porous silicon and hydrogen-terminated porous silicon with washing procedures to trap the target analytes, respectively. CHCA was used as the MALDI matrix.

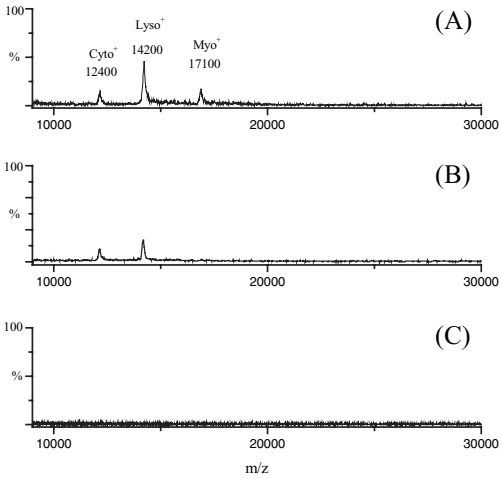

Figure 5. MALDI mass spectra for a mixture of cytochrome C (10^{-6} M), lysozyme (10^{-6} M), and myoglobin (10^{-5} M) in 100 mM buffer solution at pH of (A) 5.2, (B) 8.2, and (C) 12.2. 20 μL of sample solution was deposited on IDA-derivatized porous silicon and incubated for 10 min, vortexed for 30 s in 100 mM phosphate buffer containing 200 mM NaCl, and finally washed with deionized water. CHCA was used as the MALDI matrix.

hydrophobicity of the silicon-hydrogen bond was involved in the adsorption of the protein molecules.

The IDA-derivatized porous silicon was also used for the pretreatment of a protein solution containing 10 mM SDS. Figure 4(A) shows the MALDI mass spectrum of a solution (1 μL) of cytochrome C (10^{-5} M) and lysozyme (10^{-5} M) containing 10 mM SDS solution, deposited onto the IDA-terminated porous silicon surface. Only a weak signal for cytochrome C is observed in the mass spectrum. Figure 4(B) illustrates the MALDI mass spectrum obtained for the same sample solution (1 μL) following pretreatment on the IDA-derivatized porous silicon, and signals for cytochrome C and lysozyme can be readily detected in this case. However, when the same sample solution was deposited onto the hydrogen-terminated porous silicon surface, only a very weak cytochrome C signal was observed (Fig. 4(C)). Similarly, when the sample solution was treated on the hydrogen-terminated porous silicon surface with the same pretreatment as for the IDA-terminated porous silicon, only weak signals for cytochrome C and lysozyme were observed (Fig. 4(D)). All the results indicated that the IDA-derivatized porous silicon surface has a higher affinity capacity than the underivatized porous silicon surface and that it can be used to remove contaminants and to selectively concentrate the target molecules in a sample solution.

pH values strongly affect the selectivity for the capture of target proteins in the sample solution by IDA-derivatized porous silicon. Amphiprotic compounds, such as peptides and proteins, have unique isoelectric points (pI). When the pH of a protein sample solution is below the pI value of the protein, the protein molecules have a net positive charge. Thus negatively charged porous silicon surfaces tend to attract positively charged protein molecules through electrostatic interactions. Figure 5 shows the MALDI mass spectra for a mixture of myoglobin (10^{-5} M, pI 6.8), cytochrome C (10^{-6} M, pI 10.6), and lysozyme (10^{-6} M, pI 11.0) in sample solutions at different pH values. It can be seen that all three proteins can be readily detected at pH 5.2 (Fig. 5(A)), that only cytochrome C and lysozyme can be detected at pH 8.2 (Fig. 5(B)), and that no signals for the three proteins can be detected at pH 12.2 (Fig. 5(C)). These results suggest that with increasing pH, the net charges of protein molecules in a sample solution change from positive to negative. Therefore, the amount of the protein molecules captured by carboxylic acid groups on the porous silicon surface is gradually decreased, and no protein molecule signals can be detected at pH 12.2.

The IDA-derivatized porous silicon was used to trap target species in a real sample. The major proteins in egg white are ovalbumin, ovomucoid, globulins, and conalbumin, accounting for 54, 11, 10 and 13% of the total proteins, respectively, while lysozyme is the minor component only accounting for about 3.5%.[41,42] The egg white was first diluted 10-fold with 5 mM phosphate buffer at pH 7.0, and then further diluted 5-fold with the CHCA matrix solution to obtain a mass

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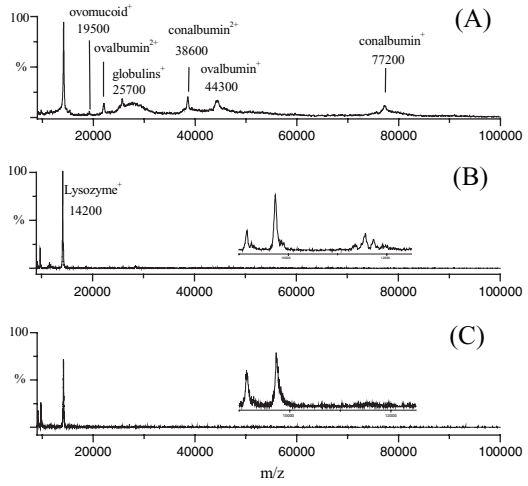

Figure 6. MALDI mass spectra of egg white solutions. (A) Direct analysis of 1 μL diluted egg white in 5 mM phosphate buffer. (B, C) Depositing 20 μL diluted egg white in 100 mM phosphate buffer at pH 7.0 and 9.0 on IDA-derivatized porous silicon, vortexing for 30 s in 100 mM phosphate buffer containing 200 mM NaCl and then washing with deionized water. CHCA was used as the MALDI matrix.

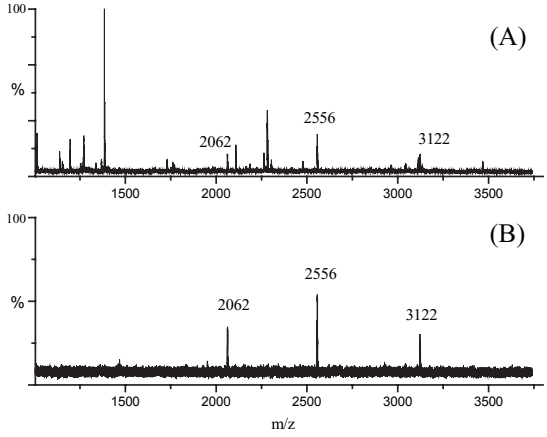

Figure 7. MALDI mass spectra of the tryptic digest of β-casein (10^{-6} M, 1 μL) (A) with direct analysis on Fe^{3+}-IDA-derivatized porous silicon surface and (B) with sample pretreatment on Fe^{3+}-IDA-derivatized porous silicon surface to selectively trap the target peptide. DHB (25 mg/mL) containing 1% H_3PO_4 was used as the MALDI matrix.

spectrum with good signal-to-noise. A typical mass spectrum of this diluted egg white acquired by conventional MALDI-MS is shown in Fig. 6(A). The mass spectrum shows seven ions at *m/z* 14200 ($lysozyme^+$), 19 500 ($ovomucoid^+$), 22 200 ($ovalbumin^{2+}$), 25 700 ($globulins^+$), 38 600 ($conalbumin^{2+}$), 44 300 ($ovalbumin^+$) and 77 200 ($conalbumin^+$). Figure 6(B) presents the mass spectrum for diluted egg white solution (20 μL) at pH 7.0 following sample pretreatment on the IDA-derivatized porous silicon. Egg white lysozyme (pI 11.1) has a net positive charge at the given pH value, whereas ovomucoid (pI 4.0), ovalbumin (pI 4.6), globulins (pI 5.5–5.8) and conalbumin (pI 6.6) have a negative charge.[42] Therefore, lysozyme and other positively charged proteins were trapped on the IDA-derivatized porous silicon, and are readily detected by MALDI-MS. When the egg white sample was processed on the IDA-derivatized porous silicon at pH 9.0, however, only lysozyme and two other unknown proteins were detected by MALDI-MS, as shown in Fig. 6(C).

The enrichment of phosphopeptides on Fe^{3+}-IDA-derivatized porous silicon for MALDI-MS analysis

Protein phosphorylation is important for the regulation of the activity of proteins and is of considerable importance in proteomics research.[43–45] Fe^{3+}-IDA-derivatized porous silicon can also be used to selectively trap phosphopeptides from the digested products of phosphoproteins. From our observations, DHB is a better matrix than CHCA for the detection of phosphopeptides in MALDI-MS. The spectra of phosphopeptides can be enhanced[44,45] when phosphoric acid is used as the acid dopant in DHB matrix. Thus DHB containing 1% H_3PO_4 was used as the MALDI matrix for the analysis of phosphopeptides in the following experiment. Three phosphopeptides digested from the phosphoprotein β-casein were detected at *m/z* 2062, 2556 and 3122, and the detailed information is listed in Table 1. Figure 7(A) shows the MALDI mass spectrum of the tryptic digest of β-casein (10^{-6} M, 1 μL) on the Fe^{3+}-IDA-derivatized porous silicon surface. Signals for the three phosphopeptides and for other non-phosphopeptides are present in the spectrum. The MALDI mass spectrum obtained when the Fe^{3+}-IDA-derivatized porous silicon surface is used as an affinity probe to selectively bind the phosphopeptides from the tryptic digest of β-casein (10^{-6} M, 1 μL) is shown in Fig. 7(B). Only the $[M+H]^+$ ions at *m/z* 2062, 2556 and 3122 for the peptides appeared in this mass spectrum. Then, 1 μL of the tryptic digest of β-casein at a concentration of 10^{-8} M was directly analyzed by MALDI-MS, and the mass spectrum is presented in Fig. 8(A). The signals for the phosphopeptides and non-phosphopeptides are almost undetectable in this spectrum. When Fe^{3+}-IDA-derivatized porous silicon was used to selectively trap the target peptides from the deposited 30 μL of the tryptic digest solution (10^{-8} M), the $[M+H]^+$ ions of the phosphopeptides at *m/z* 2062, 2556 and 3122 could still be observed in the MALDI mass spectrum, as shown in Fig. 8(B). All the above results indicate that

Table 1. Phosphopeptides trapped from the tryptic digest of β-casein on Fe^{3+}-IDA-derivatized porous silicon surface

β-Casein	$[M+H]^+$ (*m/z*)	Phosphorylated sites	Amino acid sequence
β1	2062	1	FQ[pS]EEQQQTEDELQK
β2	2556	1	IEKFQ[pS]EEQQQTEDELQDK
β3	3122	4	RELEELNVPGEIVE[pS]L[pS][pS][pS]EESITR

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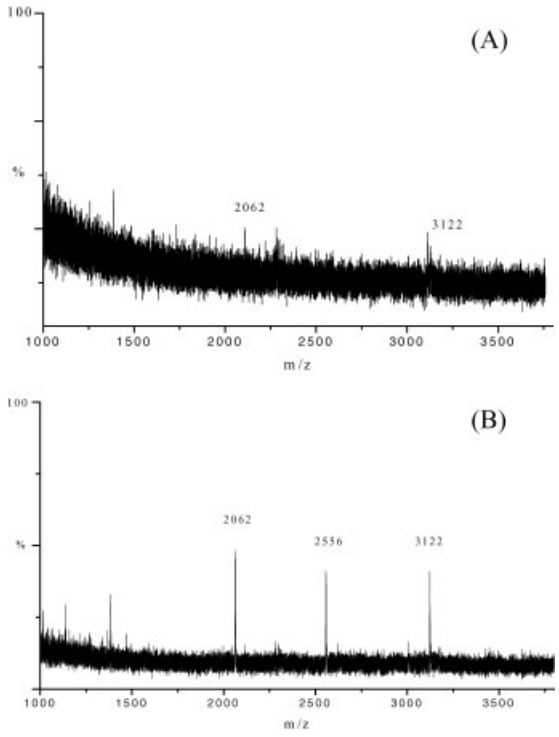

Figure 8. MALDI mass spectra of the tryptic digest products of β-casein (10^{-8} M) obtained (A) with direct analysis and (B) with sample pretreatment on Fe^{3+}-IDA-derivatized porous silicon surface to trap selectively the phosphopeptides from the deposited sample solution (30 μL). DHB (25 mg/mL) containing 1% H_3PO_4 was used as the MALDI matrix.

Fe^{3+}-IDA-derivatized porous silicon can specifically trap and effectively concentrate phosphopeptides from the digest products of phosphoproteins.

CONCLUSIONS

It was demonstrated that the IDA-derivatized porous silicon prepared through a photochemical reaction could be used to selectively trap positively charged species in sample solution through electrostatic and hydrophobic interaction. High concentrations of contaminants such as urea and surfactants can be quickly removed from protein solutions by sample pretreatment on the IDA-derivatized porous silicon before analysis by MALDI-TOFMS. The IDA-derivatized porous silicon was also employed to selectively trap lysozyme in egg white for MALDI analysis. The IDA-derivatized porous silicon can be converted into Fe^{3+}-IDA-derivatized porous silicon when needed, and the porous silicon surface obtained can specifically trap and effectively concentrate phosphopeptides from tryptic digests of phosphoproteins.

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