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Nanoparticle-single drop microextraction as multifunctional and sensitive nanoprobes: Binary matrix approach for gold nanoparticles modified with (4-mercaptophenyliminomethyl)-2-methoxyphenol for peptide and protein analysis in MALDI-TOF MS

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ABSTRACT

For the first time, we demonstrated that the nanoparticle-single drop microextraction (NP-SDME) technique can be applied as multifunctional nanoprobes to serve as the binary matrix, affinity and desalting probes for sensitive detection of peptides and proteins in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). This approach introduced a simple method to synthesis gold nanoparticles modified with (4-mercaptophenyliminomethyl)-2-methoxyphenol in toluene to serve as extraction phases in the single drop microextraction (SDME) technique. We successfully applied this multifunctional nanoprobe to extract an array of peptides and proteins at low concentration (fmol range) in MALDI-MS. In addition, for the first time, we demonstrated that the two matrix system (binary matrix) by simply mixing the organic matrix with the Au NP-SDME microdroplets, can offer high sensitivity detection of peptides and proteins. Signal intensity can be significantly enhanced up to 35-fold for a hydrophobic peptide (gramicidin D) comparing to that of the traditional organic matrix. Finally, current approach could effectively analyze milk proteins from milk samples even under high salt conditions (5 M NaCl). We believe that the interplay of nanoparticles with SDME as multifunctional probes for MALDI-MS analysis of peptides and proteins could be a powerful tool for future proteomic studies.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been widely applied for the analysis of biomolecules [1,2] such as proteins [3], nucleic acids [4], biomarkers [5–8], microorganisms [9], drugs [10,11], peptides [12] and carbohydrates [13]. However, during the MALDI-MS analysis, it is necessary to maintain the signal quality since the unwanted molecules (from major components or sample pretreatment processes of biological samples) present in sample solution often suppress the analyte signals. Difficulties are often encountered for the analysis of complex samples which are the challenges for analytical chemists. Methods of sample pretreatment have been developed for MALDI-MS analysis of biomolecules such as surface modified $CaCO_3$ [14], zeolite nanocrystals [15] and polymeric microbeads [16] have been reported for enrichment of peptides or proteins in MALDI-MS. However, these methods typically require tedious preparation or separation procedures; they are time consuming and may cause samples loss. Recently, nanomaterials including Au NPs [17], Ag NPs [18–20], Pt nanoflowers [21], SiO_2 NPs [22], Si nanopowders [23], ZnS NPs [24] and ZnO NPs [25,26] have been successfully applied in MALDI-MS as the matrix or affinity probes for analysis of proteins or peptides due to their large surface to volume ratio, small in size, and easy for chemical modification on their surfaces.

Development of various liquid phase microextraction (LPME) techniques as preconcentration probes which can be directly coupled to MALDI-MS for rapid analysis of a variety of compounds (drugs [10,27–30], surfactants [31], amino acids or peptides [32]), has become very popular because these techniques are inexpensive, easy to operate and straightforward. These techniques include micropipette extraction of LPME [27], drop to drop solvent microextraction (DDSME) [28] and single drop microextraction (SDME) [10,29–31] and reversed micellar microextraction (RMME)

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[32]. However, these solvent based extraction approaches are not sufficient for extraction of larger biomolecules such as peptides or proteins. Therefore, to overcome these problems, nanoparticle based single drop microextraction (NP-SDME) has been attracted considerable attention as a novel affinity probe for biomolecule analysis in MALDI-MS. Previous efforts for the NP-SDME coupled with MALDI-MS for rapid extraction and identification of peptides or proteins by using modified silver [18,19] and gold nanoparticles [17] have been reported. After SDME extraction to exclude the washing steps, samples in the NP-SDME microdroplets can be directly deposited on target plates for MALDI-MS analysis. These methods could significantly enhance the detection signals of peptides and proteins in MALDI-MS. In addition, direct analysis of biomolecules using NP-SDME techniques could reduce the analyte loss and to concentrate analytes into small volume (1–2 μL) of organic solvent which are most suitable for directly interfacing to MALDI-MS. Although the NP-SDME techniques can serve as effective affinity probes, they are only applied for single purpose in MALDI-MS. Therefore, in this study, we introduced a simple NP-SDME method to serve as a multifunctional nanoprobe for extraction and enrichment of low abundance of peptides and proteins in MALDI-MS. We synthesized the Au NP modified with (4-mercaptophenyliminomethyl)-2-methoxyphenol prepared in toluene and successfully applied it as a binary matrix, affinity and desalting probe for sensitive biomolecule analysis in MALDI-MS.

2. Experimental

2.1. Chemicals

High quality of chemicals (>98%) and solvents (>99%) was used for synthesis of nanoparticles; vanillin, 4-aminothiophenol, sodium borohydride ($NaBH_4$), gold chloride ($AuHCl_4{\bullet}3H_2O$), α-cyano-4-hydroxycinnamic acid (CHCA), sinapic acid (SA), toluene, ethanol, acetic acid, and all peptides or proteins such as insulin, ubiquitin, HW6, cytochrome *c*, lysozyme, myoglobin and gramicidin D, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Milk (1% fat) was purchased from local market of Kaohsiung, Taiwan. The water used for all experiments was purified by a Milli-Q water system (Millipore, Milford, MA, USA).

2.2. Synthesis of (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3)

Vanillin, ethanol, acetic acid and 4-aminothiophenol were used for the synthesis of (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3). Briefly, 5 mL of ethanol and 250 mg of vanillin were added into a round-bottom flask (25 mL). Then, one drop of acetic acid was added into the flask under magnetic stirring; 205 mg of 4-aminothiophenol was added into the flask and stirred for 5 h at room temperature. The reaction product (solid powder) was washed with cold ethanol and then dried in the air at room temperature.

2.3. Synthesis of the modified gold nanoparticles in toluene

The modified gold NPs were prepared by reduction of $AuHCl_4{\bullet}3H_2O$ solution with $NaBH_4$. Briefly, 0.5 mL of 0.01 M of $NaBH_4$ was added dropwise to 100 mL of 0.29 mM $AuHCl_4{\bullet}3H_2O$ solution. The solution turns colorless after addition of the $NaBH_4$ solution. Then, the prepared gold in aqueous solution was added to the toluene solution containing 1.66 mM (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3) and the solution was stirred vigorously for 5 h. The Au NP was successfully transferred to the organic phase (toluene), as indicated by the color change from light yellow to dark brown. Then, the brown color of the Au NP in toluene layer was separated by a funnel and then washed with deionized water for several times.

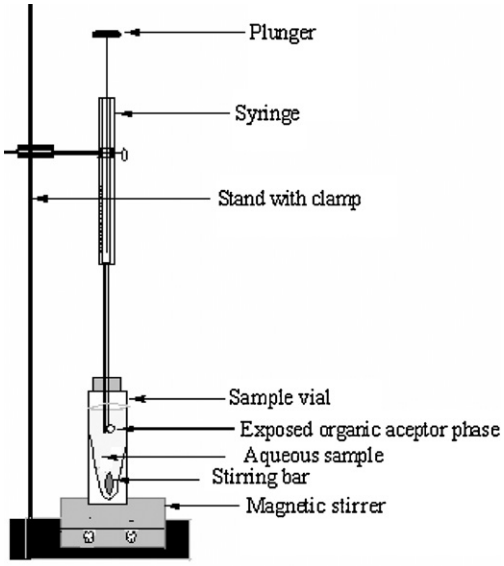

Fig. 1. Experimental setup of Au NP-SDME.

2.4. Procedure for lysozyme analysis from milk

Lysozyme from milk samples (1% fat) was extracted according to [33]. A 5 mL aliquot of milk sample and 15 mL of NaCl (1 M) solution were taken in a 50 mL of beaker. The pH of the sample solution was adjusted to 6 using 1N of NaOH solution. Then, the mixture was stirred at 40 °C for 1 h. After that, the pH of the solution was further adjusted to 2.2 by addition of 1N of HCl and kept for 15 min at room temperature. Finally, the solution was filtered with a quantitative filter paper (No. 6, hole diameter 11 μm, thickness 0.18 mm, and diameter 70 mm, Tokyo, Japan). The filtrate obtained from milk was used for the analysis of lysozyme.

2.5. Procedure for milk protein analysis under high salt concentration

Milk proteins were extracted from milk samples according to the following procedure [34]. A 5 mL aliquot of milk and 15 mL of sodium chloride solution (5 M) were placed in a 50 mL beaker. Then the mixture was stirred at 40 °C for 1 h. Finally, the solution was filtered with a quantitative filter paper and then the filtrate was used for NP-SDME/MALDI-MS analysis under optimized conditions. To perform the NP-SDME, 50 μL of milk sample was dissolved in 1 mL of deionized water (aqueous solution) and then we performed the NP-SDME extraction as in Fig. 1.

2.6. Extraction procedures of NP-SDME

In the NP-SDME technique, to extract the analytes from an aqueous solution, a microdrop of organic solvent is placed on the tip of the microsyringe and immersed into aqueous solution containing analytes (see Fig. 1). The NP-SDME experiments were performed for the following procedures. Peptides and proteins with desired concentrations were spiked into a glass vial filled with 1 mL ample solution. A 10 μL of microsyringe (Hamilton Co., Reno, Nevada, USA) was used to draw 1 μL of toluene containing the modified Au NPs. The microsyringe was inserted into the sample solution through a PTFE-coated silicon septum of screw cap of a glass vial. As soon

as the sample was extracted into the 1 μL microdroplet of organic solvent (the modified Au nanoparticles prepared in toluene), the microdroplet was drawn back into the microsyringe and then directly placed the 1 μL microdroplet onto the target plates for subsequent MALDI-MS analysis.

2.7. UV–vis, Fourier transform-infrared (FT-IR) and SEM measurements

The UV–vis absorption spectrum of (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3) capped Au NPs prepared in toluene was measured by using an UV-vis spectrophotometer (model: U-3501, Hitachi, Tokyo, Japan). The FT-IR spectrometer (model: IFS-48, Bruker, Bremen, Germany) was applied for identification of functional groups on the surface modification of (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3) capped Au NPs. The morphology of the modified Au NPs was confirmed by using a scanning electron microscope (SEM), model: JEOL 6700F, Tokyo, Japan.

2.8. MALDI-TOF MS analysis

All mass spectra were acquired by using a MALDI time-of-flight (TOF) mass spectrometer (Microflex, Brucker Daltonics, Bremen, Germany) equipped with an accelerating voltage 20 kV and a nitrogen laser (337 nm). All detection parameters were set the same as previous conditions [24].

3. Results and discussion

3.1. Characterization of the modified Au NPs

The gold nanoparticles capped with (4-mercaptophenyliminomethyl)-2-methoxyphenol was synthesized by reaction of vanillin (1) with 4-aminothiophenol (2) in the presence of catalytic quantity of acetic acid (AcOH) and absolute ethanol as solvent at room temperature under constant stirring. The obtained

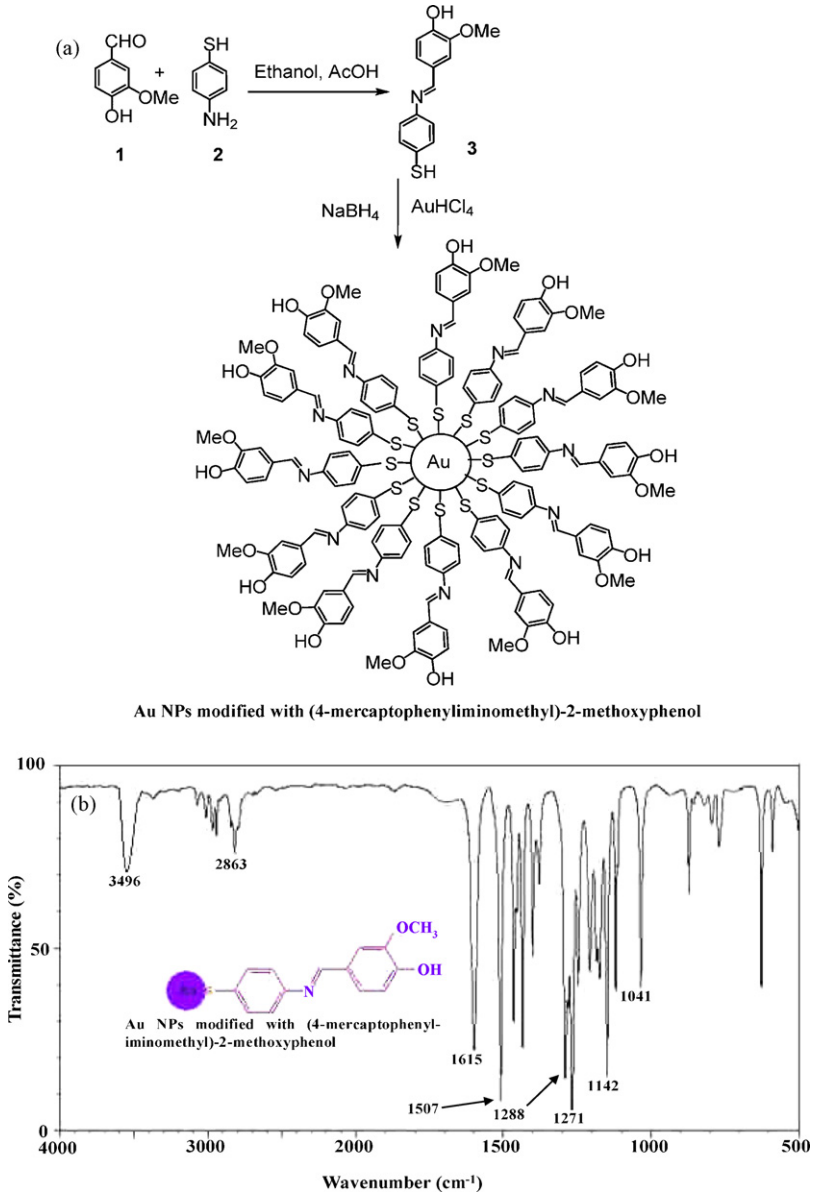

Fig. 2. (a) Schematic representation of synthesized gold nanoparticles capped with (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3) and (b) FT-IR spectra of gold NPs modified with (4-mercaptophenyliminomethyl)-2-methoxyphenol (Schiff base 3).

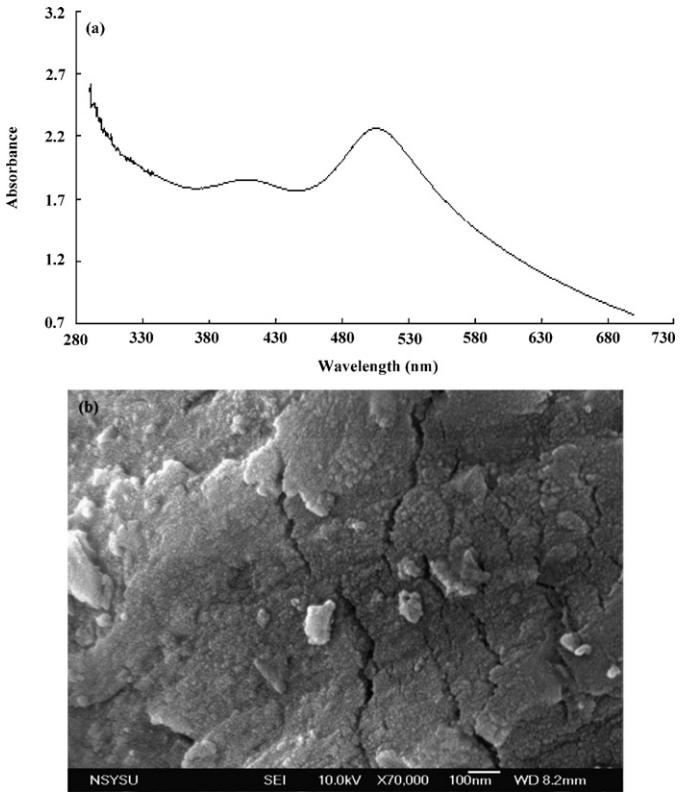

Fig. 3. (a) UV–vis absorption spectrum of gold NPs modified with (4-mercaptophenyliminomethyl)-2-methoxyphenol and (b) SEM image of modified gold NPs.

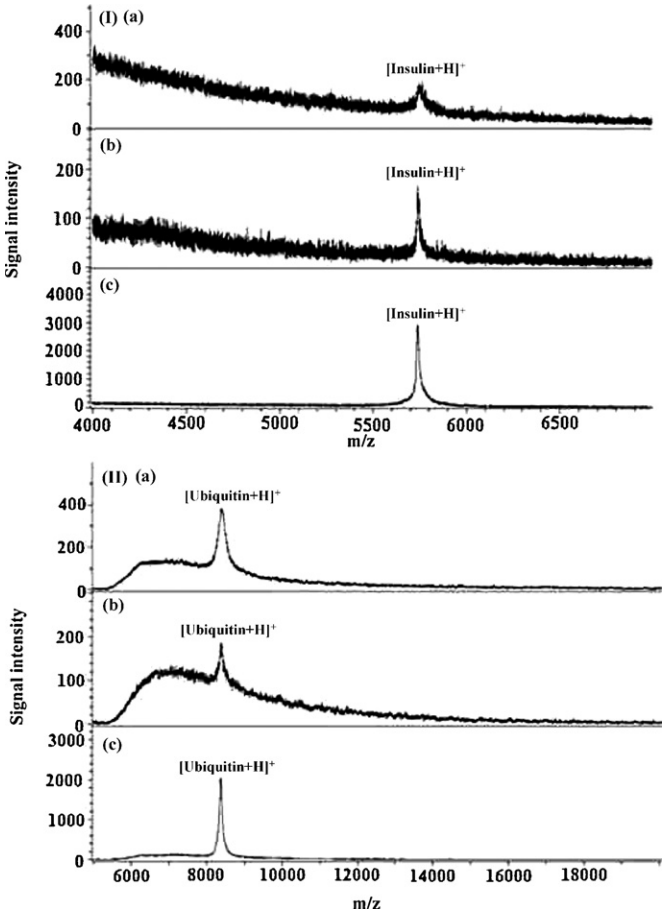

Fig. 4. MALDI-TOF mass spectra for analysis of (I) insulin (17.4 fmol) of (a) using Au NP-SDME as the matrix, (b) direct analysis using CHCA (5000 ppm) as the matrix and (c) binary matrix approach using Au NP-SDME along with CHCA (5000 ppm). The laser energy was 76.4 μJ. (II) Ubiquitin (23.3 fmol) of (a) direct analysis using CHCA (5000 ppm) as the matrix, (b) Au NP-SDME as the matrix and (c) binary matrix approach using Au NP-SDME with CHCA (5000 ppm). The laser energy was 73.2 μJ. All mass spectra were generated at 200 pulsed laser shots and the *m*/*z* values of mass peaks are listed in Table 1.

product was commonly named as Schiff base (3). The synthesis procedures and structure of the functionalized gold NPs from Schiff base are shown in Fig. 2a. The modified Au NPs prepared in toluene were produced by reaction of Schiff base (3) with gold nanoparticles which were obtained from reduction of $AuHCl_4 \bullet 3H_2O$ by using $NaBH_4$ as reducing agent in aqueous solution and then phase transfer to toluene. The modified Au NPs with (4-mercaptophenyliminomethyl)-2-methoxyphenol were confirmed by the FT-IR (Fig. 2b), UV–vis (Fig. 3a) and SEM (Fig. 3b). The FT-IR spectrum clearly shows the absence of thiol (–SH) group stretching band around 2650–2780 cm^{-1}; this indi-

Table 1
Comparison of Au NP–SDME technique with conventional MALDI-MS methods (using organic matrices) for peptide/protein analysis.

Name of analytes, ions	Au NP-SDME as the matrix		Au NP-SDME as the binary matrix		Organic matrices (CHCA/SA)	
	m/*z* value	Signal intensity	*m*/*z* value	Signal intensity	*m*/*z* value	Signal intensity
Gramicidin D, $[Val\text{-}GA+H]^+$	1883	110	1883	810	–	–
Gramicidin D, $[Val\text{-}GA+Na]^+$	1905	323	1905	2185	1905	68
Gramicidin D, $[Val\text{-}GA+K]^+$	1921	138	1921	2143	–	–
HW6, $[HW6+H]^+$	906	925	906	6852	906	1121
Insulin, $[Insulin+H]^+$	5743	195	5743	3200	5743	198
Ubiquitin, $[Ubiquitin+H]^+$	8404	191	8403	2077	8404	389
Cytochrome *c*, $[Cytochrome\ c+H]^+$	–	–	12,387	186	–	–
Lysozyme, $[Lysozyme+H]^+$	–	–	14,300	91	–	–
Myoglobin, $[Myoglobin+H]^+$	–	–	16,962	177	16,961	38
[a]Lysozyme, $[M+H]^+$	–	–	14,355	385	14,355	30
[a]Proteoso pep. PP81, $[M+H]^+$	–	–	9168	258	9168	20
[a]γ_3-Casein, $[M+H]^+$	–	–	11,498	215	–	–
[a]α-Lactoalbumin, $[M+H]^+$	–	–	14,218	1050	14,218	10
[a]β-Lactoglobulin, $[M+H]^+$	–	–	18,394	175	–	–

"–", no observation of ions or signals.
[a] Generated from milk.

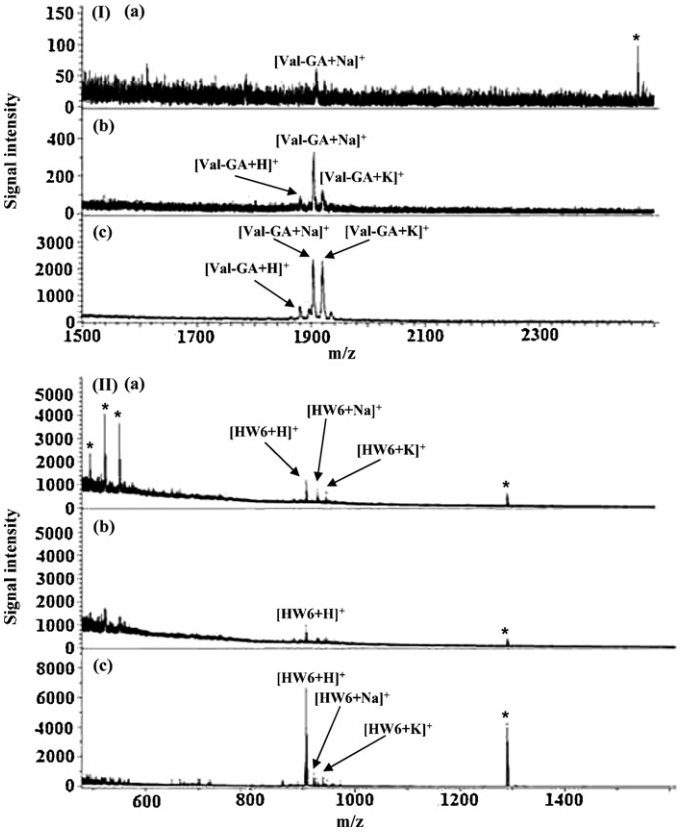

Fig. 5. MALDI-TOF mass spectra for analysis of (I) gramicidin D (53.1 fmol) of (a) direct analysis using CHCA (5000 ppm), (b) Au NP-SDME as the matrix and (c) binary matrix approach using Au NP-SDME with CHCA (5000 ppm). The laser energy was 65.4 μJ. (II) HW 6 (450 fmol) of (a) direct analysis using CHCA (5000 ppm), (b) Au NP-SDME as the matrix and (c) binary matrix approach using Au NP-SDME with CHCA (5000 ppm). The laser energy was 60.7 μJ. All mass spectra were generated at 200 pulsed laser shots and the *m*/*z* values of mass peaks are depicted in Table 1. *The background noises derived from the organic matrix (CHCA).

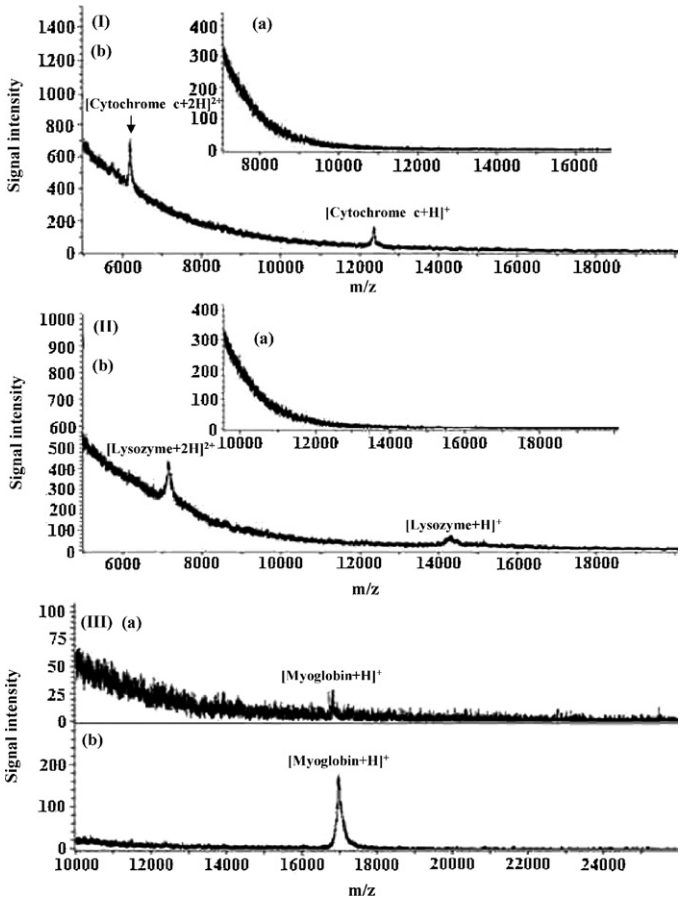

Fig. 6. MALDI-TOF mass spectra for analysis of (I) cytochrome *c* (40 fmol) (a) direct analysis using SA (5000 ppm) as the matrix and (b) Au NP-SDME along with SA (5000 ppm) as the binary matrix. (II) Lysozyme (13.2 fmol) (a) direct analysis using SA (5000 ppm) as the matrix and (b) Au NP-SDME with SA (5000 ppm) as the binary matrix. (III) Myoglobin (35.3 fmol) (a) direct analysis using SA as the matrix and (b) Au NP-SDME along with SA (5000 ppm) as the binary matrix. The laser energy was 64.4 μJ. All mass spectra were generated at 200 pulsed laser shots and the *m*/*z* values are listed in Table 1.

cates that the Schiff base was successfully coupled with gold NPs by the formation of new bond between Au and Schiff base (Au–S). The stretching vibration band at 1615 cm^{-1} represents azomethine group (–CH=N) and the stretching band at 3496 cm^{-1} is due to the hydroxyl group (–OH). The band around 2863 cm^{-1} is due to hydrogen of azomethine carbon (–CH=N). The maximum UV–vis absorption wavelength of the modified Au NP was observed at 525 nm (Fig. 3a) and it confirms the formation of Au NPs. The above results indicate that the Schiff base was successfully coupled with Au NPs, which is more suitable for the analysis of biomolecules in complex mixtures by SDME and subsequent identification with MALDI-TOF MS.

3.2. Determination of the optimal conditions for the NP-SDME/MALDI-MS technique

From previous work of NP-SDME [17,19], we carefully considered the optimal conditions for the Au NP-SDME/MALDI-MS technique such as selection of extraction solvent, extraction time, sample agitation rate and matrix conditions. We found that the optimal conditions for the same parameters which can be applied to all peptides and proteins in this study were: sample agitation rate 200 rpm, extraction time 30 min, toluene as extraction solvent and to perform the extraction at room temperature. The reasons have been discussed in previous work [17,19]. While the other parameters such as matrix conditions, matrix concentration and laser energy required to be examined carefully for each analyte. The number of laser shots for each spectrum was collected at a constant number (200 laser shots) in order to fairly evaluate the detection sensitivity from comparison of the (absolute) intensity of mass spectra for all analytes. The effect of extraction time was investigated for extraction of insulin (as a model compound) from aqueous solution for the NP-SDME/MALDI-MS technique at various time intervals (1, 10, 30 and 50 min) and the results were shown in Fig. S1 of Supporting Information. The best extraction efficiency of insulin by using Au NP-SDME/MALDI-MS was shown at 30 min of extraction time (Fig. S1c). When it was compared with 10 and 50 min of extraction time (Fig. S1b and d of Supporting Information), about 33–50-fold of signal enhancement could be achieved. While at 1 min of extraction time, we did not observe any signal. Thus, we applied 30 min as the optimal extraction time for further experiments.

3.3. Binary matrix approach by using Au NP-SDME extraction of peptides and proteins in MALDI-MS

The Au NP-SDME technique was examined for the functions to serve as a matrix and an affinity probe for the analysis of various peptides and proteins in MALDI-MS and the results are displayed in Figs. 4–7. All experiments were performed under optimal parameters of NP-SDME for the following conditions: sample agitation rate 200 rpm, toluene as extraction solvent extraction

time 30 min and the extraction was performed at room temperature. Other parameters such as matrix conditions and laser energy were carefully investigated for each individual experiment. Fig. 4(I) shows the results for detection of insulin (17.4 fmol) from aqueous solution via Au NP-SDME/MALDI-MS by comparing using Au NP-SDME as the matrix (Fig. 4(I)a), CHCA as the matrix (Fig. 4(I)b) and binary matrix approach by using Au NP-SDME with CHCA (Fig. 4(I)c). We found that although all these three methods can generate insulin spectra, only the binary matrix approach (Fig. 4(I)c) can produce nice and clean spectra for free of background interferences and high signal-to-noise ratio and the signal intensity was significantly enhanced for 15 times compared with the other two methods. Fig. 4(II) displays the spectra for detection of ubiquitin (23.3 fmol) from aqueous solution through Au NP-SDME/MALDI-MS by comparing using CHCA as a matrix (Fig. 4(II)a), Au NP-SDME as the matrix (Fig. 4(II)b) and binary matrix approach by using Au NP-SDME with CHCA (Fig. 4(II)c). Although all these three methods can generate successful spectra, only the binary matrix approach can produce nice and clean spectra for free of background interferences and high signal-to-noise ratio and the signal intensity was significantly enhanced for 5 and 10 times compared with those of using CHCA and Au NP-SDME as the matrix, respectively. In addition, we successfully extracted small peptides such as gramicidin D (representing a hydrophobic peptide [12]) and HW6 (representing a thio peptide) by using Au NP-SDME/MALDI-MS and the obtained spectra were shown in Fig. 5. Again, the binary matrix approach can produce nice and clean spectra for free of background interferences and high signal-to-noise ratio for gramicidin D (Fig. 5(I)c) and HW6 (Fig. 5(II)c) too. The signal intensity was significantly enhanced for 4–35 times for gramicidin D and HW6 compared with those of using CHCA and Au NP-SDME as the matrix. Note that the Au NP-SDME is especially sensitive for gramicidin D detection and this may be attributed to the Au NP exhibits hydrophobic nature and thus can greatly enhance the detection sensitivity for this hydrophobic peptide to 35 times when compared with direct analysis by using CHCA. Thus, we know that hydrophobic and thio peptides can be successfully generated by using binary matrix approach in the Au NP-SDME/MALDI-MS. Furthermore, the present method can be applied for the extraction of low concentration of high mass proteins such as cytochrome *c* (40 fmol), lysozyme (13.2 fmol) and myoglobin (35.3 fmol) and the results are shown in Fig. 6. For these three proteins with high mass between 12,000 and 16,000 Da, it was very difficult to succeed detect the mass peaks at low concentration for these proteins when using SA as the conventional matrix. However, when we applied binary matrix approach of Au NP-SDME/MALDI-MS to detect the above proteins, they were successfully detected at fmol concentration. Furthermore, current approach also can be successfully applied to extract protein mixtures (insulin and ubiquitin) from aqueous solution (Fig. S2 of Supporting Information) and the *m*/*z* values are listed in Table 1.

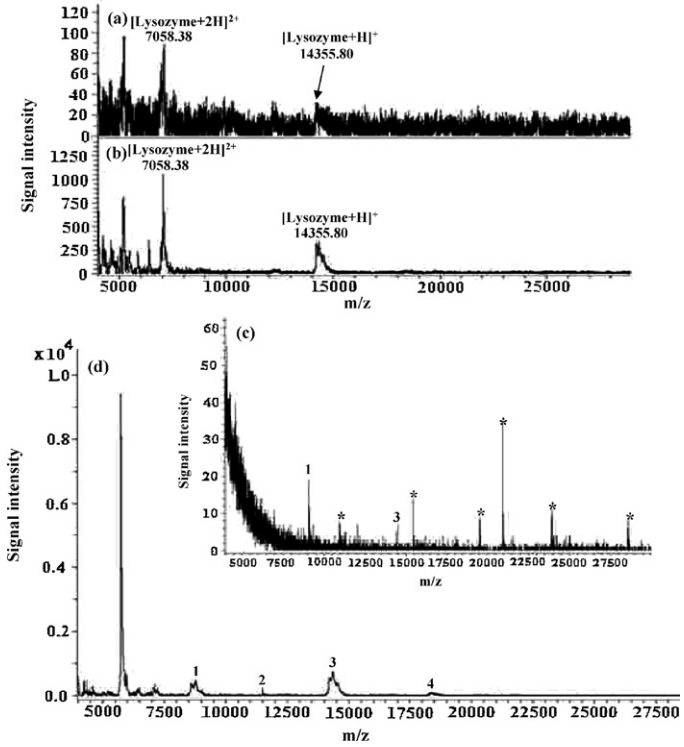

Fig. 7. MALDI-TOF mass spectra of analysis of milk samples (50 μL) for (a) lysozyme analysis via direct analysis using SA as the matrix (b) lysozyme analysis via Au NP-SDME with SA as the binary matrix (c) milk protein analysis via direct analysis using SA as the matrix in the presence of 5 M NaCl (d) milk protein analysis via Au NP-SDME with SA as the binary matrix in the presence of 5 M NaCl. The milk proteins were identified as (1) proteoso pep. PP81, (2) γ_3-casein, (3) α-lactoalbumin and (4) β-lactoglobulin. The laser energy was 56.8 μJ and SA concentration was 5000 ppm. All mass spectra were generated at 200 pulsed laser shots. *The background noises derived from the organic matrix (SA).

3.4. Application of the Au NP-SDME/MALDI-MS on real word sample analysis under high salt conditions

To demonstrate the applicability of the Au NP-SDME/MALDI-MS technique can be applied to analysis real world samples under high salt interferences, we have performed the milk protein extraction via Au NP-SDME/MALDI-MS from milk solution and the results are shown in Fig. 7. Fig. 7a shows the direct analysis of lysozyme from milk samples using conventional MALDI-MS method (SA as the matrix). The detection of lysozyme from milk was extremely difficult by the conventional MALDI-MS method. However, after the binary matrix approach using Au NP-SDME extraction, the identification of lysozyme from milk can be easily detected (Fig. 7b). This confirms that the binary matrix approach of Au NP-SDME was superior for the extraction of lysozyme from milk samples than that of conventional MALDI-MS method (using SA as the matrix). Next, we further demonstrated the binary matrix capability of Au NP-SDME technique for the extraction of a variety of milk proteins under high salt conditions (in the presence of 5 M NaCl) by comparison of conventional MALDI-MS method (Fig. 7c) with the binary matrix approach (Fig. 7d). Comparison of both spectra, the binary matrix approach using Au NP-SDME was able to identify four milk proteins than those of conventional MALDI-MS method (SA as the matrix) because only two protein ions were detected while many intensive interference peaks were observed in Fig. 7c. In Fig. 7d, the generated milk proteins at *m*/*z* 9168.01, 11,498.10, 14,218.10 and 18,394.56 were assigned as proteoso pep. PP81 (1), γ_3-casein (2), α-lactoalbumin (3) and β-lactoglobulin (4), respectively [34]. From the above results, we found that the binary matrix approach of Au NP-SDME can effectively detect the real world samples even under high salt conditions.

3.5. The advantage of the Au NP-SDME nanoprobe for MALDI-MS

Current approach introduced a simple, straightforward and effective method to improve the detection sensitivity of peptide and proteins in MALDI-MS. The binary matrix approach applying Au NP-SDME microdroplets which can homogeneously mixed with the organic matrix (CHCA or SA) to form homogeneous crystals and thus favorable for sensitive detection of peptides and proteins at low concentration (fmol) in MALDI-MS. In addition, the Au NP-SDME can also serve as multifunctional nanoprobes for ionization, enrichment, preconcentration and desalting purposes for a

variety of peptides and proteins. After the SDME extraction procedures, the sample can be directly deposited onto the MALDI target plates and directly sent for MALDI-MS analysis without the need for any further washing steps or elution processes. Thus, sample loss can be avoided. Additionally, the current approach can be useful for separation and rapid analysis of peptides and proteins at low concentration in MALDI-MS. Because the Au NP was modified organic compounds with aromatic structures, which is favorable for UV absorption of laser light and thus facilitate the efficient energy transfer to analytes resulting in sensitive detection of peptides and proteins in the MALDI-MS. Additionally, the modified Au NP-SDME/MALDI-MS approach was compared with conventional MALDI-MS method (direct analysis by using organic matrices) as shown in Table 1. The results in Table 1 clearly demonstrated that the Au NP-SDME/MALDI-MS technique is a highly sensitive preconcentrating technique for peptide/protein analysis from complex mixtures.

4. Conclusion

For the first time, we successfully introduced a simple method to synthesize the modified Au NP in toluene which can detect peptides and proteins at low concentration (fmol range) and to serve as multifunctional nanoprobes in the MALDI-MS. The Au NP-SDME nanoprobe can be efficiently applied as binary matrix, preconcentration probe, affinity probe and desalting probe in MALDI-MS simultaneously. Besides, we have successfully demonstrated that the analysis of proteins from real world samples (milk). Therefore, current approach is a simple, sensitive and straightforward technique for simultaneous preconcentration, enrichment, desalting and rapid identification of peptides and proteins from complex samples in the MALDI-MS.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.01.065.

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