Talanta 82 (2010) 540–547

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Electrostatically self-assembled azides on zinc sulfide nanoparticles as multifunctional nanoprobes for peptide and protein analysis in MALDI-TOF MS

Hui-Fen Wu[a,b,c,*], Suresh Kumar Kailasa[a,b], Lokesh Shastri[a]

[a] Department of Chemistry, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan
[b] Center for Nanoscience and Nanotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan
[c] Doctoral Degree Program in Marine Biotechnology, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan

ARTICLE INFO

Article history:
Received 18 March 2010
Received in revised form 6 May 2010
Accepted 8 May 2010
Available online 16 May 2010

Keywords:
$ZnS-N_3$ NPs
Peptides
Proteins
Microwave enzymatic digestion
MALDI-TOF MS

ABSTRACT

A simple method to synthesize electrostatically self-assembled azides on zinc sulfide nanoparticles ($ZnS-N_3$ NPs) was described and then it was further applied as a multifunctional nanoprobe such as enriching, desalting, accelerating and separation-/washing free nanoprobes for rapid analysis of peptides and proteins and microwave assisted tryptic digested proteins in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The $ZnS-N_3$ NPs were characterized by UV–vis, FT-IR, SEM and TEM spectroscopy. The $ZnS-N_3$ NPs can effectively enrich signal intensities for 2–10 times for various peptides and proteins including HW6, insulin, ubiquitin, cytochrome c, lysozyme, myoglobin and bovine serum albumin (BSA) in MALDI-TOF MS. Furthermore, we also demonstrated that the $ZnS-N_3$ NPs can serve as accelerating probes for microwave assisted tryptic digestion of proteins in MALDI-TOF MS. The applicability of the present method on complex sample analysis such as milk proteins from cow milk and ubiquitin and ubiquitin like proteins from oyster mushroom were also demonstrated.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was introduced in the year of 1988 by Karas and Hillenkamp and it has been widely applied in the fields of biomedical and biological science [1,2]. It is a powerful tool for the analysis of various peptides and proteins [3–5]. Recently, MALDI-MS has been widely applied for clinical diagnosis [6], bacteria detection [7] and discovery of biomarkers for various bacterial characterizations [8,9]. However, the detection of biomolecules in complex mixtures in MALDI-MS is still a difficult task. Therefore, various NP-based MALDI-MS approaches have been reported to improve the detection sensitivity and enrichment of peptides and phosphoproteins with zeolite and magnetic beads [10,11]. Additionally, various efforts have been directed for efficient separation and enrichment of peptides and proteins at low concentration in MALDI-MS. For example, Fang et al. [12] applied immobilized metal ion affinity chromatography (IMAC) using nanozeolite composite as the sorbent for the separation and analysis of proteins. This affinity based technique has become a powerful tool for the analysis of various molecules in MALDI-MS. In addition, polymeric microbeads were utilized as concentrating probes for selective trapping of tryptic digested proteins in MALDI-MS [13]. C_8- and C_{60}-functionalized magnetic materials have been applied for separation and enrichment of peptides and proteins from biological samples in MALDI-MS [11,14–16]. Other sample pretreatment techniques such as surface modified $CaCO_3$ [17], zeolite nanocrystals [18] and polymeric microbeads [13] were also demonstrated for biomolecule analysis. Recently, extensive efforts have been focused on applying nanomaterials as the matrices [19,20] or affinity probes [21] in MALDI-TOF MS to enhance the detection sensitivity and ionization efficiency of peptides/proteins with time consuming washing steps especially for protein digests [13,17,18]. Although nanoprobe-based mass spectrometry have been shown to be capable for the enrichment of biomolecules, to date, there is no report on using electrostatically self-assembled molecules on semiconductor NPs as affinity and accelerating probes for biomolecule analysis in the MALDI-MS. Surface modification of NPs with organic molecules has been applied as matrices in MALDI-MS for the analysis of biomolecules with increased ion yields [22]. Hence, self-assembled organic molecules modified on NP surface is an alternative method to increase the capability of NPs and to solve many difficulties in MALDI-MS such as signal enhancement, background noise suppression and accelerating the identification of peptides and proteins. Most of the reported methods have been focused on modified NPs with mercapto groups which containing methyl group ($-CH_3$), carboxylic group (–COOH) or amino group ($-NH_2$) on selected metallic or semiconductor NPs [22,23].

* Corresponding author at: Department of Chemistry, National Sun Yat-Sen University, 70, Lien-Hai Road, Kaohsiung 80424, Taiwan.
Tel.: +886 7 5252000x3955; fax: +886 7 525 3908.
E-mail address: hwu@faculty.nsysu.edu.tw (H.-F. Wu).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.05.026

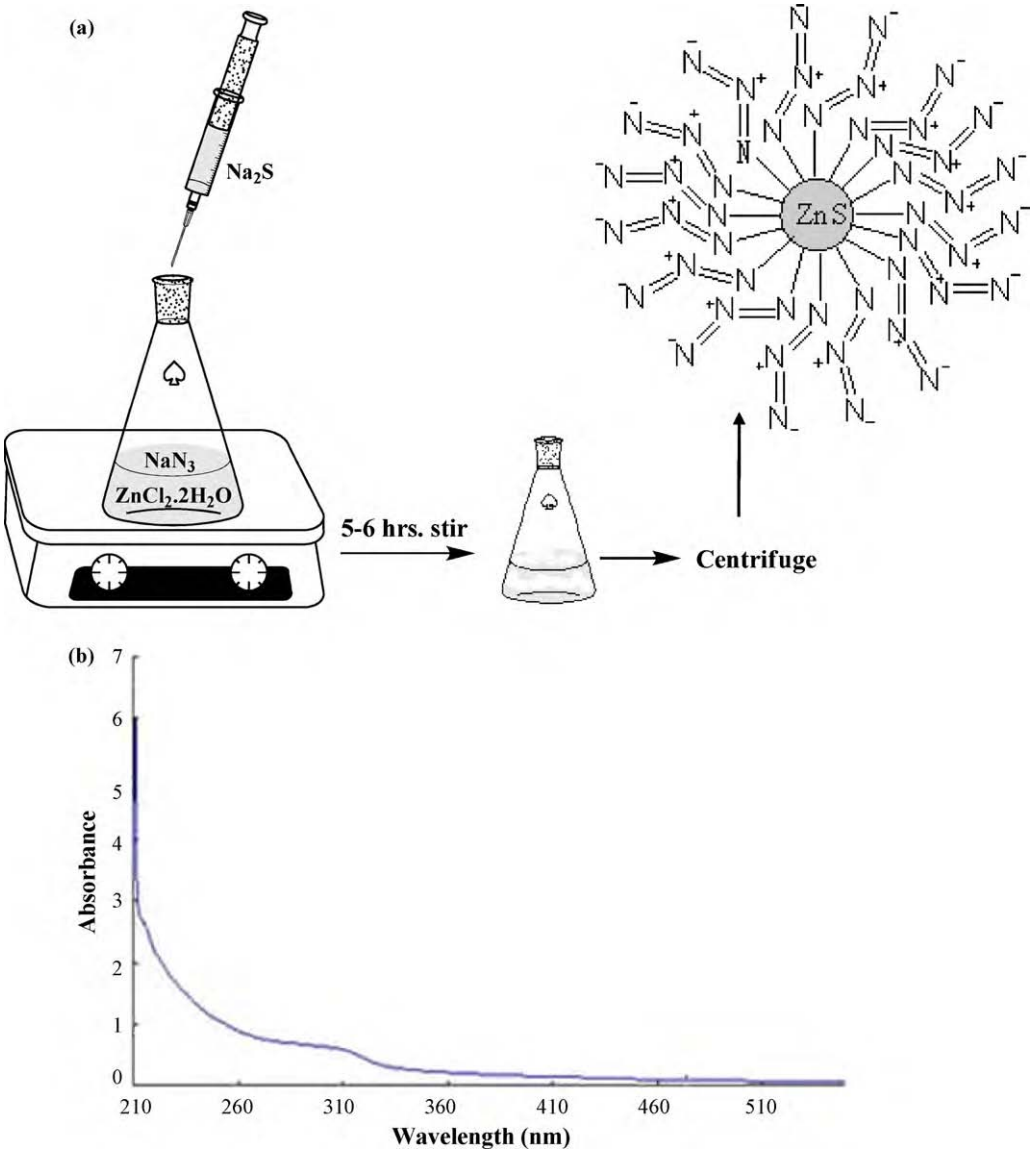

Fig. 1. (a) Schematic presentation of synthesis procedure for ZnS-N_3 NPs and (b) UV–vis absorption spectrum of ZnS-N_3 nanoparticles.

These types of functionalized surfaces could effectively improved signal intensity and ionization efficiency of analytes in MALDI-MS. Moreover, molecular engineering is an interdisciplinary area where the supramolecular systems can be designed with potential applications. The primary objective is to design and synthesize suitable building blocks with novel and potential use for the analysis of biomolecules by mass spectrometry. Therefore, in this study, we introduced a simple method to synthesize novel surface modified ZnS NPs to serve as multifunctional nanoprobes for rapid peptide/protein analysis in MALDI-TOF MS. ZnS NPs modified with electrostatically self-assembled azide can serve as multifunctional nanoprobes including desalting, enriching, accelerating and separating/washing free nanoprobes for protein/peptide and microwave assisted tryptic digestion of proteins in MALDI-TOF MS analysis.

2. Experimental methods

2.1. Reagents and materials

Sodium sulfide was obtained from Nihon Shiyaku Industries Ltd., Japan. Sodium azide was purchased from Janssen Chimica, USA. Leucine-enkephalin (Leu-enk) and HW6 (a thiopeptide, H-HCKFWW-OH) were purchased from Kelowna International Scientific Inc., Taiwan. Sinapinic acid (SA), α-cyano-4-hydroxycinnamic acid (CHCA), dithiothreitol (DTT), zinc chloride, insulin (from bovine pancreas), ubiquitin (from bovine red cells), cytochrome c and bovine serum albumin (BSA) and lysozyme (form chicken egg white) were obtained from Sigma (St. Louis, MO, USA). The vortex agitator (VM 2000, Digi System Laboratory, Taipei, Taiwan) was used for incubation of samples. The microwave oven was purchased from LG Electronics (Taipei, Taiwan). The maximum power and the operating frequency of the microwave oven were 700 W and 2450 MHz, respectively. The water was purified by a Milli-Q ultrapure water system (Millipore, Milford, MA, USA) for the experiments. All reagents used were of analytical grade.

2.2. Preparation of peptide/protein solutions

Stock solutions of Leu-enk (1.8 mM), HW6 (1.1 mM) and insulin (0.17 mM) were prepared by dissolving 1 mg/mL in deionized water. Ubiquitin (0.12 mM), cytochrome c (78.1 μM), lysozyme (68.9 μM) and BSA (1.5 μM) were prepared in 50 mM of NH_4HCO_3 solution (1 mg/mL for each peptide). The above stock solutions were

further diluted for desired concentration (HW6, 1.0 pM; insulin, 0.25 pM; ubiquitin, 1 pM; cytochrome c, 5 pM; lysozyme, 2 pM and BSA, 5 nM). The matrix solutions of CHCA (26 mM) and SA (50 mM) were prepared using water and acetonitrile (1:2, v/v) containing 0.1% of trifluoroacetic acid (TFA).

2.3. Synthesis of azide modified ZnS nanoparticles

Although this is the first time to synthesize ZnS-N_3 nanoparticles modified with azide, the idea was associated with the literature [24,25] and the synthesis procedure is shown in Fig. 1a. Surface modification of ZnS NPs was achieved using sodium azide as the stabilizer. ZnS-N_3 NPs were prepared by mixing sodium azide solution (40 mM) with $ZnCl_2$ solution (40 mM) in a 250 mL flask. Then sodium sulfide solution was added very slowly to the above solution via a microsyringe and then stirred for 5–6 h. The resulting product was centrifuged and then the obtained solid was washed with deionized water and then dried under vacuum. The obtained ZnS-N_3 NPs were dissolved in 1 M sodium hydroxide solution and then used for MALDI-MS experiments without any further treatment.

2.4. Procedure for the analysis of biomolecules using ZnS-N_3 NPs as affinity probes in MALDI-TOF MS

Standard solutions of peptides or proteins were taken into 1 mL polyethylene vials and diluted with deionized water to desired concentration. Then, 1.7 μM of ZnS-N_3 NPs solution was added into the above solution and incubated for 1 h at room temperature. After the incubation, the vial was taken out and 0.5 μL of sample solution was placed onto the MALDI target plate followed by adding 0.5 μL of CHCA or SA solution, and further air-dried for 20 min. Then, the target plate was loaded into the MALDI source for desorption/ionization of analytes.

2.5. Procedure for microwave assisted tryptic digestion of proteins

Microwave digestions of proteins (cytochrome c and lysozyme) were performed in the Eppendorf tubes. Proteins (4 μM, 500 μL) were mixed with 4 μM of trypsin (25 μL; weight ratio from 1:1 to 50:1) in 50 mM of NH_4HCO_3 solution at pH 8. To this, 5 μL of $CaCl_2$ (1.8 M) was added into the above solution to prevent autolysis of trypsin. We added 2 μL of DTT solution (10 mM) to the above mixture to reduce the formation of disulfide bonds in proteins. After that, the ZnS-N_3 NP (1.7 μM) solution was added into the above solutions and mixed well. The solutions were put into a microwave heating for 30–50 s to digest proteins. The temperature of the sample solutions was measured immediately by a thermocouple (Genechian industrial Ltd., Taiwan). After microwave irradiation, the solutions were taken out from the microwave oven and then directly deposited onto the MALDI target plates for detection.

2.6. Procedure for milk protein analysis

Milk proteins were analyzed according to the reported method [26]. Briefly, 5 mL of milk was centrifuged at 3000 rpm for 5 min, sample was dried under N_2 gas and the dried sample was dissolved in deionized water. After that, 900 μL of above milk solution was transferred into a 1 vial (1 mL) containing 100 μL of ZnS-N_3 NPs (1.7 μM) solution and the mixture was further vortexed for 1 h. The sample vial was centrifuged at 3000 rpm for 5 min and 0.5 μL of the isolated NP-target species was directly mixed with 50 mM of SA and evaporated on the MALDI plate and then analyzed by MALDI-MS.

2.7. Procedure for isolation of ubiquitin like proteins from oyster mushroom

Ubiquitin like proteins were isolated from oyster mushroom by the described procedure [27]. Briefly, fresh fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) (100 g) were purchased from local market (Kaohsiung, Taiwan), cutting into small pieces and homogenized with 10 mM of Tris–HCl (pH 7.2). The resulting mixture was kept at 4 °C for 3 h and then the sample was centrifuged, and the supernatant solution was diluted (2-fold) with deionized water. To this, 100 μL of ZnS-N_3 NPs (1.7 μM) solution was added and then vortexed for 1 h. The target species conjugated NPs were analyzed by the above procedure.

2.8. Instrumentation

The size and morphology of ZnS-N_3 NPs were confirmed by using a scanning electron microscope (SEM; JEOL 6700F, Tokyo, Japan) and a transmission electron microscope (TEM; JEOL-3010, Tokyo, Japan). UV–vis absorption spectrum of ZnS-N_3 NPs was measured on a double-beam spectrophotometer (U-3501, Hitachi, Tokyo, Japan). ZnS-N_3 NPs were further verified by using a FT-IR spectrophotometer (PerkinElmer 100, USA).

All mass spectra were generated in the positive ion mode using a MALDI-time-of-flight mass spectrometer (Microflex, Bruker Daltonics, Bremen, Germany); 337 nm of a nitrogen laser was used for irradiation of the analytes. The accelerating voltages were set at 20 kV. All mass spectra (200 laser shots for per spectrum) were generated in the reflectron mode for peptides or in the linear mode for proteins and other parameters not mentioned here were set the same as those described previously [23]. All experiments were carried out at least three times in order to check the reproducibility of the current approach.

3. Results and discussion

3.1. Characterization of the ZnS-N_3 nanoparticles

A simple approach for synthesis of the ZnS NPs capped with azide group ($\bar{N}{=}N^{+}{=}N^{-}$) is shown in Fig. 1a, which is electrostatically self-assembled on the surfaces of ZnS NPs. The UV–vis absorption spectrum of ZnS-N_3 NPs is shown in Fig. 1b and the maximum absorption wavelength was observed at 310 nm. The FT-IR spectra of sodium azide and ZnS-N_3 NPs are presented in Fig. 2a,b. The strong band at 2113 cm^{-1} arises due to sodium azide (Fig. 2b). The FT-IR spectrum of ZnS-N_3 NPs (Fig. 2a) shows bands at 1012 and 2102 cm^{-1} are the characteristics of S–N stretching vibration and azide stretching band of ZnS-N_3 NPs, respectively. By comparing FT-IR spectra of sodium azide (Fig. 2b) and ZnS-N_3 NPs (Fig. 2a), the azide band was slightly shifted to lower wavenumber which is due to variation in the vibrational transition and the higher occupation of excited rotational levels which enhance mobility of the species leading to a different distribution in NPs system [28]. The morphology and size of ZnS-N_3 NPs were confirmed by using SEM and TEM (Fig. 2c and d). The SEM and TEM images demonstrate the uniform distribution of the NPs and size of the NPs was about 15 nm which is obtained from the histograms of ZnS-N_3 NPs (Fig. S1 of Supporting Information). The above results indicate the conclusive evidence to successfully synthesize the electrostatically self-assembled azide molecules on the ZnS NP surfaces.

3.2. ZnS-N_3 nanoparticles to serve as affinity probes for peptide/protein analysis

The first function of ZnS-N_3 NPs is to serve as affinity probes for the enrichment of peptides and proteins including HW6, insulin,

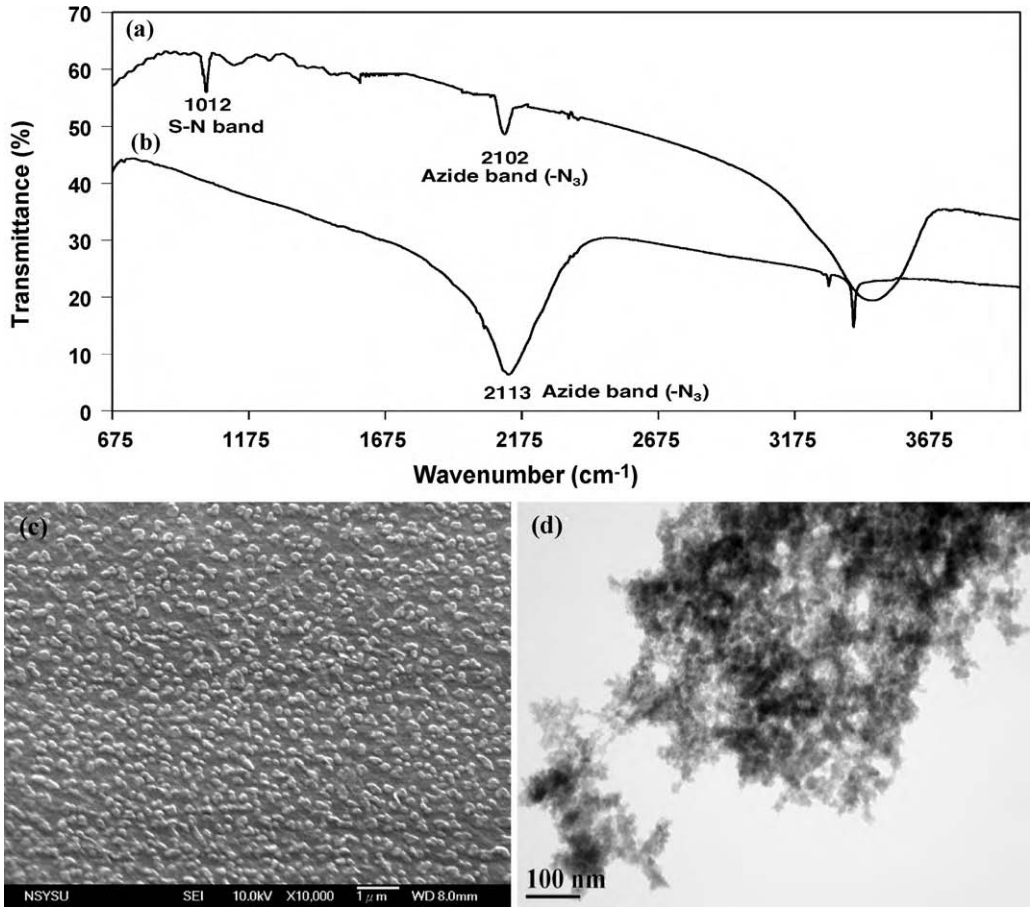

Fig. 2. FT-IR spectra of (a) ZnS-N_3 NPs and (b) free sodium azide. SEM (c) and TEM (d) images of ZnS-N_3 NPs.

ubiquitin, cytochrome c, lysozyme and BSA in MALDI-TOF MS and the results are present in Figs. 3 and 4 and Supporting Information of Fig. S2. Fig. 3a compares the direct analysis of cytochrome c (Fig. 3a(i)) with the result of using ZnS-N_3 NPs as affinity probes (Fig. 3a(ii)). We observed 7.8-fold of signal enhancement by using ZnS-N_3 NPs as affinity probes. The reason may be due to the interactions of ZnS-N_3 NPs with cytochrome c. The interactions play a significant role to improve the detection sensitivity of target analytes in MALDI-TOF MS [23]. Because the surface modification of ZnS-N_3 NPs with azide (functional group) allows for efficient interaction with peptides and proteins, the signal intensity of cytochrome c was greatly improved with ZnS-N_3 NPs as affinity probes than that of the conventional method (direct analysis) of MALDI-MS. In addition, we also applied ZnS-N_3 NPs as affinity probes for enrichment of HW6 (Fig. 3b), insulin (Fig. 3c and Fig. S2 of Supporting Information), lysozyme (Fig. 4a), ubiquitin (Fig. 4b) and BSA (Fig. 4c). The mass peaks were observed at m/z ~12,362.6, 906.3, ~5739.9, ~14,317.2, ~8384.8, ~66,351.0 and ~33,037.2 which are assigned as the protonated molecules ($[M+H]^+$) of cytochrome c, HW6, insulin, lysozyme, ubiquitin, BSA and $[M+2H]^{2+}$of BSA, respectively. From Figs. 3–4 and Figs. S2 and S5b–c of Supporting Information, comparing to the organic matrices, the ZnS-N_3 NPs served as affinity probes has demonstrated 2–10-fold of signal enhancements for peptides and proteins analysis in the MALDI-MS. From these results, we found that the functionalized ZnS NPs modified with azide group could be served as an universal affinity probe for enrichment of all tested peptides and proteins in MALDI-TOF MS. It is well known that protein or peptide interactions are mainly depending on pI values [29]. Generally, proteins (insulin pI 5.5; ubiquitin pI 6.79; cytochrome c pI 10.2; lysozyme pI 10.5 and BSA pI 4.7) or peptides (Len-enk pI 5.5 and HW6 pI 8.2) exhibit net positive or negative charges at lower or higher isoelectricpoint points (pI = pH). While the azide ($\bar{N}{=}N^{+}{=}N^{-}$) functional group carries both positive and negative charges, which is able to bind efficiently with charged species of peptides/proteins through electrostatic attractions without changing pH of the sample solution.

We also found that the mass to charge ratios of proteins were slightly varied in the generated mass spectra. This is due to mass independent (initial) velocities of ions [30] or the broad distribution of the initial kinetic energy [31]. To further demonstrate the trapping efficiency of the current method for larger biomolecules, we also examined the enrichment experiments for high mass protein (BSA) and the result is shown in Fig. 4c in which the protonated molecules of BSA were successfully enriched by using the ZnS-N_3 NPs as affinity probes. Note that the increase in intensity of $[M+H]^+$ (singly protonated BSA ions) and decrease in $[M+2H]^{2+}$ ions when using ZnS-N_3 NPs as affinity probes (SA as the matrix) (Fig. 4c(ii)). The reason may be due to the following. (1) The addition of NPs facilitates the adsorption of BSA onto the NPs leading to the reduction of the possible reactive sites for formation of the doubly protonated BSA ions. (2) The micro-heterogeneity of organic matrix crystals might be able to reduce the adduct formation of the ions generated from the organic matrix crystals [32,33].

3.3. ZnS-N_3 nanoparticles to serve as accelerating probes for microwave assisted tryptic digestion of proteins

The second function of ZnS-N_3 NPs is to serve as the accelerating probes in microwave assisted enzymatic digestion of proteins.

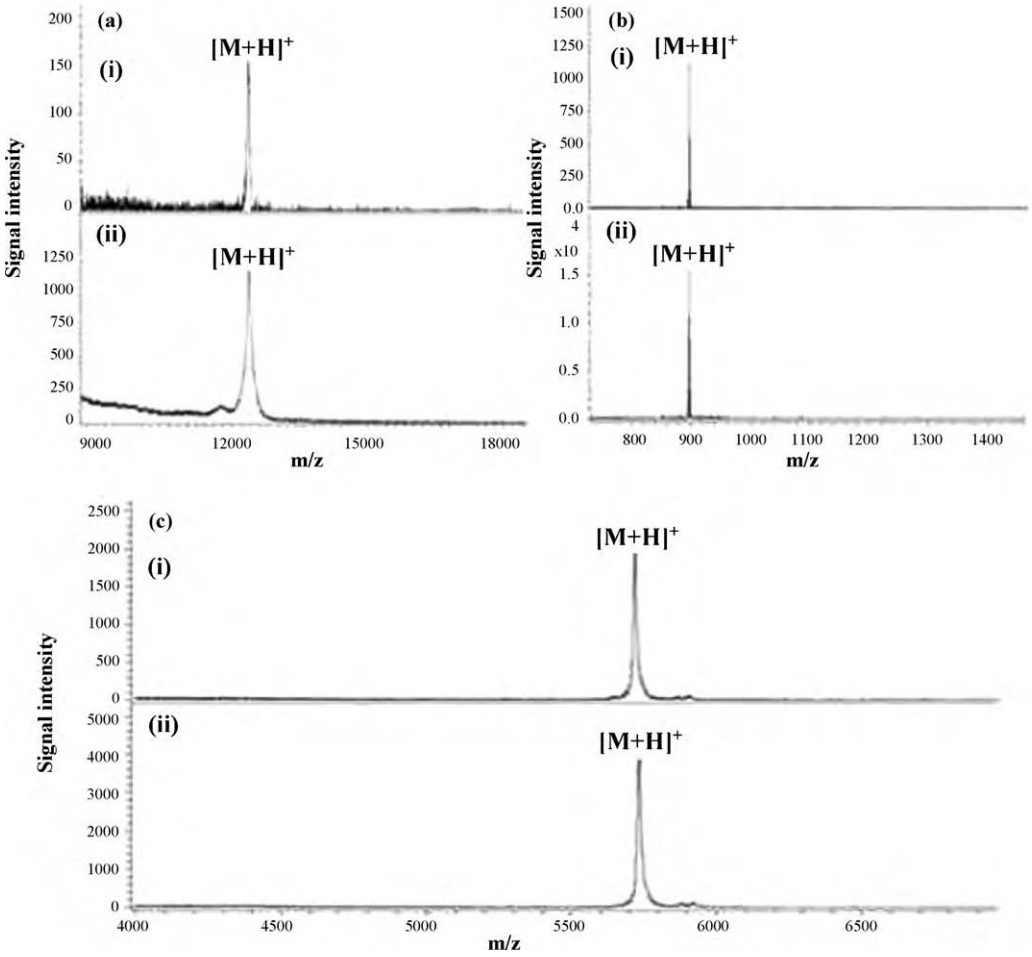

Fig. 3. (a) MALDI-TOF mass spectra of cytochrome c (5 pM) using SA (50 mM) as the matrix: (i) direct analysis and (ii) using ZnS-N_3 NPs (1.7 μM) as affinity probes. The mass peak of protonated cytochrome c was shown at m/z ~12,362.6. (b) MALDI-TOF mass spectra of HW6 (1 pM) using CHCA (26 mM) as the matrix: (i) direct analysis and (ii) using ZnS-N_3 NPs as affinity probes and the protonated mass peak of HW6 was observed at m/z 906.3. (c) MALDI-TOF mass spectra of insulin (0.25 pM) using CHCA as the matrix (26 mM): (i) direct analysis and (ii) using ZnS-N_3 NPs (1.7 μM) as affinity probes and mass peak was observed at m/z ~5739.9. The samples were incubated 1 h for HW6 and insulin, 2 h for cytochrome c at room temperature. The mass spectra were generated at 76.3 μJ of laser energy.

The results are shown in Fig. 5 and Supporting Information of Figs. S3 and S4. Fig. 5a contrasts the mass spectra of microwave assisted tryptic digestion of cytochrome c (2 pM) via direct analysis (without ZnS-N3 NPs) (Fig. 5a(i)) with that of using ZnS-N_3 NPs (Fig. 5a(ii)). Fig. 5b compares the mass spectra of microwave assisted tryptic digestion of lysozyme (2 pM) through direct analysis using CHCA as the matrix (without ZnS-N_3 NPs) (Fig. 5b(i)) with that of using ZnS-N_3 NPs as accelerating probes (Fig. 5b(ii)). It can be noticed that without ZnS-N_3 NPs in microwave digestion (Fig. 5a(i) and b(i)), only five peptide fragments of cytochrome c and lysozyme were observed with low intensity under the intense background interferences. After the treatment of ZnS-N_3 NPs in microwave experiments, the digested fragments of cytochrome c and lysozyme were effectively enriched and much clean spectra (with less interferences) were observed by using ZnS-N_3 NPs as accelerating probes and mass spectra are shown in Fig. 5a(ii) and b(ii). The identified sequences of digested cytochrome c and lysozyme are shown in Tables S1 and S2, respectively. The sequences of digested proteins were further identified by searching from http://www.expasy.org/tool/peptide-mass.html. This reveals that ZnS-N_3 NPs can function as effective accelerating probes for microwave tryptic digestion of proteins. Next, we also compared the capability of ZnS-N_3 NPs as heat absorbers for microwave assisted tryptic digestion of proteins by measuring the temperature of protein solution with and without treatment of ZnS-N_3 NPs under microwave heating for 50 s. We found that the temperature of sample solution was $47.2 \pm 0.1\,^{\circ}C$ without ZnS-N_3 NPs. After using ZnS-N_3 NPs as accelerating probes in the microwave experiments, the temperature of solution was increased to $59.3 \pm 0.2\,^{\circ}C$. We also optimized the microwave heating time (10, 30, 50 and 60 s) for efficient microwave assisted tryptic digestion of cytochrome c and lysozyme. We found that at 30 and 50 s of microwave irradiation time, more number of digested protein signals were observed by using ZnS-N_3 NP as accelerating probes. However, only very few digested proteins were observed at 30 s of microwave heating (see Figs. S3 and S4 of Supporting Information). The maximum number of peaks of digested proteins were observed at 50 s of microwave heating time. Hence, we selected 50 s as the optimal (microwave) heating time for efficient tryptic digestion of cytochrome c and lysozyme.

3.4. *ZnS-N_3 nanoparticles to serve as desalting probes for protein analysis*

The third function of ZnS-N_3 NPs is to serve as the desalting probes of proteins in MALDI-MS analysis. We studied the capability of ZnS-N_3 NPs as affinity probes for direct analysis of insulin by MALDI-TOF MS from high salt solution (1.0–6.0 M of NaCl). The results indicated that the ZnS-N_3 NPs can successfully generate mass spectrum of insulin at high salt conditions up to 4.0 M NaCl

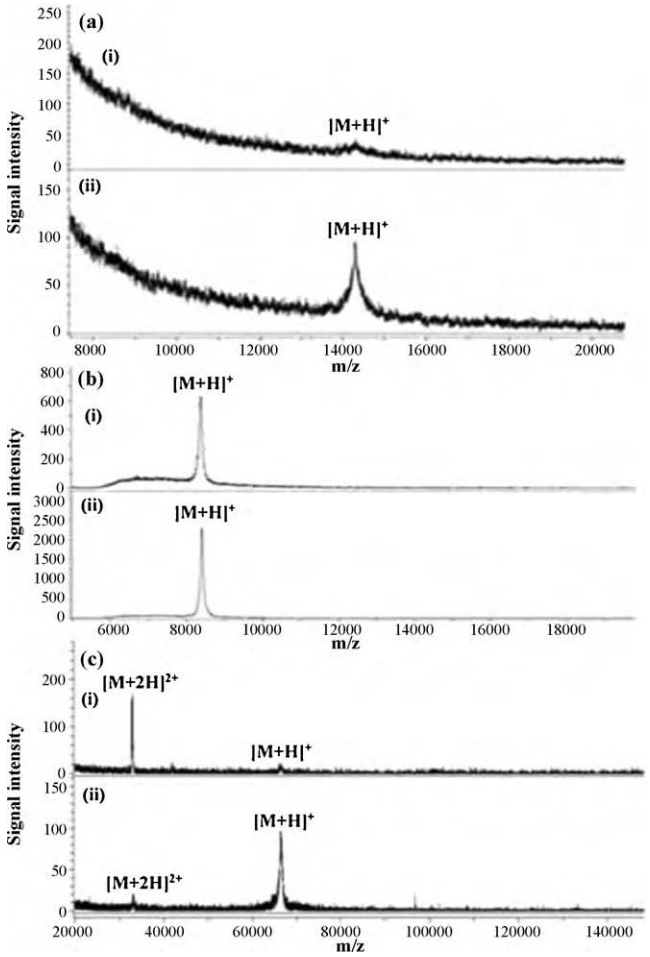

Fig. 4. (a) MALDI-TOF mass spectra of lysozyme (2 pM) using SA (50 mM) as the matrix: (i) direct analysis and (ii) with ZnS-N_3 NPs as affinity probes. (b) MALDI-TOF mass spectra of ubiquitin (1 pM) with CHCA (26 mM) as the matrix: (i) direct analysis and (ii) using ZnS-N_3 NPs as affinity probes. (c) MALDI-TOF mass spectra of BSA (5 nM) with SA (50 mM) as the matrix: (i) direct analysis and (ii) using ZnS-N_3 NPs as affinity probes. The mass peaks of the proteins were observed at m/z ~14,317.3, ~8384.8, ~66,351.0 and ~33,037.2 which are corresponding to $[M+H]^+$ ions of lysozyme, ubiquitin, BSA and $[M+2H]^{2+}$ of BSA, respectively. The proteins were incubated 1 h for ubiquitin, 2.0 h for lysozyme and BSA at room temperature. The mass spectra were generated at laser energy of 76.3 μJ for lysozyme and ubiquitin, and 80.2 μJ for BSA.

(see Fig. S5a of Supporting Information). When we applied salt concentration more than 4.0 M NaCl, the insulin signals were drastically decreased. This indicates that ZnS-N_3 NPs exhibit excellent capability to act as effective desalting probes for protein analysis in high salt interferences in MALDI-TOF MS.

3.5. Application of ZnS-N_3 nanoparticles for analysis of complex samples

We demonstrated the powerful applicability of ZnS-N_3 nanoparticles for the direct (separation-/washing free) analysis of proteins from complex samples by MALDI-TOF MS. We analyzed two real samples: (1) milk proteins from cow milk and (2) ubiquitin and ubiquitin like proteins from oyster mushroom. Fig. 6a compares the mass spectra of milk proteins detected from milk samples without (Fig. 6a(i)) and with ZnS-N_3 NPs as affinity probes (Fig. 6a(ii)) using SA as the conventional matrix. The mass peaks shown in Fig. 6a(ii) were identified at m/z 9108.8, 1159.8, 14,230.9, 14,310.3 and 18,395.0, which are corresponding to proteoso peptide (1), y_3-casein (2), α-lactalbumin (3), lysozyme (4) and β-lactoglobulin (5), respectively [34]. These results clearly indicate that ZnS-N_3 NPs are able to directly detect the milk proteins from complex samples. Next example is to directly analyze ubiquitin and ubiquitin like proteins from oyster mushroom by MALDI-TOF MS. The yield of ubiquitin and ubiquitin like proteins in oyster mushroom is 100–230 μg/kg (fruiting bodies). Hence, it is necessary to develop a simple, sensitive and straightforward method for direct detection of ubiquitin and ubiquitin like proteins in mushroom by MALDI-TOF MS. We successfully applied ZnS-N_3 NPs as affinity probes for rapid and direct identification of ubiquitin and ubiquitin like proteins from oyster mushroom in Fig. 6b(ii). Fig. 6b(i) displays the direct analysis of ubiquitin proteins extracted from oyster mushroom by using SA (50 mM) as the matrix. Comparing Fig. 6b(i) with b(ii) which was obtained using ZnS-N_3 NPs as affinity probes along with SA as the matrix, the mass signals of ubiquitin and ubiquitin like proteins were significantly enhanced (more than 4-fold). The obtained mass peaks at m/z 8062 (8.0 kDa), 8382 (8.3 kDa), 8509 (8.5 kDa) and 9165 (9.1 kDa) are corresponding to the ubiquitin and ubiquitin like proteins, respectively. Fig. 6b(iii) represents the MALDI mass spectrum of standard ubiquitin using ZnS-N_3 NPs as affinity probes along with SA as the matrix. The above results indicated that the current approach is an effective tool for separating/washing free analysis of proteins from complex samples (or biological samples) in the MALDI-TOF MS.

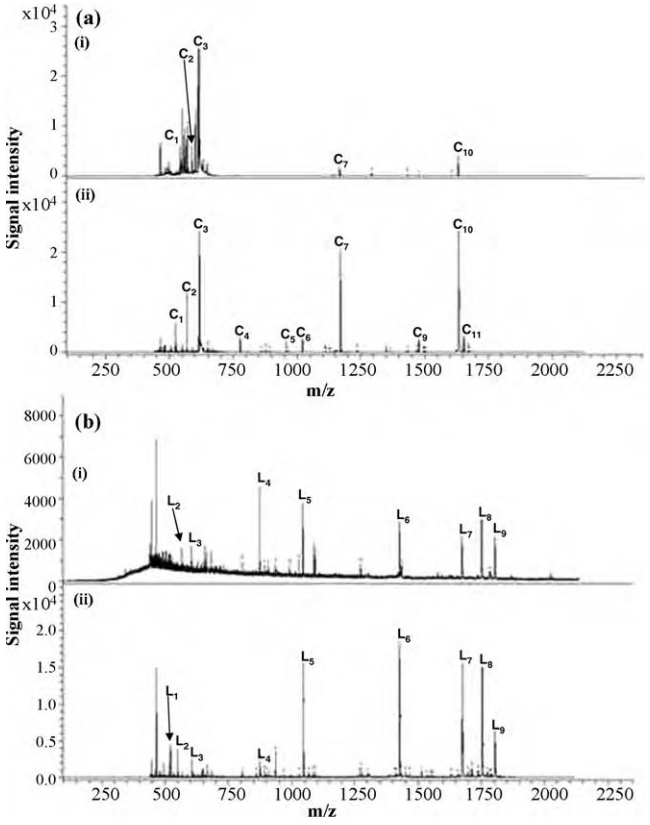

Fig. 5. (a) MALDI-TOF mass spectra of microwave assisted tryptic digestion of cytochrome c (2 pM) using CHCA (26 mM) as the matrix: (i) direct analysis and (ii) using 1.7 μM of ZnS-N_3 NPs as accelerating probes. (b) MALDI-TOF mass spectra of microwave assisted tryptic digestion of lysozyme (2 pmol) with CHCA (26 mM) as the matrix via (i) direct analysis and (ii) using 1.7 μM of ZnS-N_3 NPs as accelerating probes. The samples were radiated in microwave for 50 s and the mass spectra were generated at 49.7 μJ of laser energy.

3.6. Advantages of multifunctional ZnS-N_3 nanoprobes for MALDI-MS

Based on the above results, ZnS-N_3 NPs exhibit many advantages. (1) It can be well dispersed and stabilized in aqueous solution.

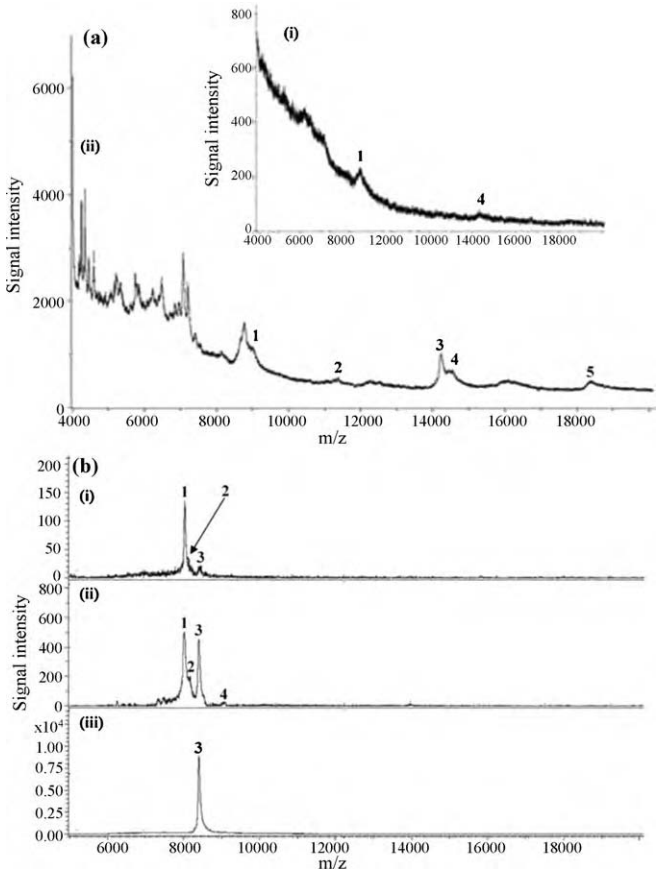

Fig. 6. (a) MALDI-TOF mass spectra for the analysis of milk proteins from cow milk (100 μL) using SA (50 mM) as the matrix via (i) direct analysis and (ii) using ZnS-N_3 nanoparticles as affinity probes. The generated mass peaks assigned at *m/z* 9108.8, 1159.8, 14,230.9, 14,310.3 and 18,395.0 are corresponding to proteoso peptide (1), y_3-casein (2), α-lactalbumin (3), lysozyme (4) and β-lactoglobulin (5), respectively. (b) MALDI-TOF mass spectra of ubiquitin and ubiquitin like proteins from oyster mushroom using SA as the matrix (50 mM): (i) direct analysis, (ii) using ZnS-N_3 NPs (1.7 μM) as affinity probes, and (iii) standard ubiquitin using ZnS-N_3 NPs (1.7 μM) as affinity probes. The mass peaks 1, 2, 3 and 4 are representing for *m/z* 8062 (8.0 kDa), 8382 (8.3 kDa), 8509 (8.5 kDa) and 9165 (9.1 kDa) which corresponds to ubiquitin and ubiquitin like proteins, respectively. The samples were incubated for 1 h at room temperature. The mass spectra were generated at 59.6 μJ of laser energy.

Thus it can be homogeneous mixed with the organic matrix to form homogeneous crystals for efficient detection of target analytes in MALDI-MS. (2) It is an universal nanoprobe for ionization for a variety of peptides and proteins by elimination of background interferences in MALDI-MS. To confirm this point, we compared small peptide (leucine-enkephalin (MW = 556.2)) with (Fig. S5b of Supporting Information) and without (Fig. S5c of Supporting Information) using ZnS-N_3 NPs as affinity probes and CHCA as the matrix. This results indicate that the background signals were successfully reduced by applying ZnS-N_3 NPs as affinity probes and the mass peaks were generated with high intensity of sodium and potassium adduct ions of Leu-enk (Fig. S5b of Supporting Information). While direct analysis using CHCA as the conventional matrix, suffered from intense interference peaks originated from CHCA clusters (see Fig. S5c of Supporting Information). However, when we used ZnS-N_3 NPs as affinity probes along with CHCA system was provided efficient platform for the analysis of small peptides with suppressed background signals. This reason might be due to CHCA is conjugated with NPs/analyte system to form polymeric NP surfaces which can drastically reduce matrix interference and background noise in the low mass range [35]. Meanwhile, the large surface area of the nanoparticle can boost-up laser desorption and ionization of target analytes. (4) The ZnS-N_3 NP solution can be stable at room temperature for several months. (5) The ZnS-N_3 nanoparticles were synthesized without using any surfactants which could avoid adduct formation or wanted interferences produced from protein–surfactant interaction, surfactant aggregates or interferences from surfactants. Thus, this approach can be useful for the rapid analysis of the peptides, proteins and digested proteins in MALDI-MS. The significance of the current method is attributed to the strong electrostatic interactions between ZnS-N_3 NPs (due to dipolar charges on the azide stabilizer units) with biomolecules (peptides or proteins).

4. Conclusion

For the first time, we successfully introduced a simple approach to synthesis ZnS-N_3 NPs capped with azide, which is able to enhance the detection sensitivity of peptides and proteins and to serve as accelerating probes for microwave assisted tryptic digestion of proteins in MALDI-TOF MS. This is because ZnS-N_3 NPs possess the unique feature of carrying both positive and negative charges on the azide functional groups. We also successfully demonstrated for the analysis of milk proteins from cow milk and ubiquitin and ubiquitin like proteins from oyster mushroom. The current approach is a simple, sensitive and straightforward analytical tool for enriching, desalting, rapid and direct identification of peptides and proteins in biological samples in MALDI-TOF MS.

Acknowledgements

We thank National Science Council of Taiwan for financial support and Dr. Suresh Kumar is grateful to SVNIT, Surat, India for permission of leave for the research work at National Sun Yat-Sen University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.05.026.

References

[1] M. Karas, F. Hellenkamp, Anal. Chem. 60 (1988) 2299–2301.
[2] R.F. Service, Science 310 (2005) 1132–1134.
[3] A.E. Ashcroft, Nat. Prod. Rep. 20 (2003) 202–215.
[4] W. Pusch, J.H. Wurmbach, H. Thiele, M. Kostrzewa, Pharmacogenomics 3 (2002) 537–548.
[5] L.H. Cohen, A.I. Gusev, Anal. Bioanal. Chem. 373 (2002) 571–586.
[6] W. Pusch, M. Kostrzewa, Curr. Pharm. Des. 11 (2005) 2577–2591.
[7] M. Hercules, A.R. Woolfitt, M.G. Carvalho, A. Pavlopoulos, L.M. Teixeira, G.A. Satten, J.R. Barr, FEMS Immunol. Med. Microbiol. 53 (2008) 333–342.
[8] V. Ruelle, E.M. Benaissa, Z. Willy, L. Philippe, D.P. Edwin, Rapid Commun. Mass Spectrom. 18 (2004) 2013–2019.
[9] M. Pignone, K.M. Greth, J. Cooper, D. Emerson, J. Tang, J. Clin. Microbiol. 44 (2006) 1963–1970.
[10] Y. Zhang, X. Wang, W. Shan, B. Wu, H. Fan, X. Yu, T. Yi, P. Yang, Angew. Chem. Int. Ed. 44 (2005) 615–617.
[11] N. Yao, H. Chen, H. Lin, C. Deng, X. Zhang, J. Chromatogr. A 1185 (2008) 93–101.
[12] X. Fang, Y. Wang, X. Wang, Y. Zhang, Y. Tang, P. Yang, Adv. Mater. 15 (2003) 1751–1753.
[13] A. Doucette, D. Craft, L. Liang, Anal. Chem. 72 (2000) 3355–3362.
[14] K. Sparbier, T. Wenzel, M. Kostrzew, J. Chromatogr. B 840 (2006) 29–36.
[15] H. Chen, X. Xu, N. Yao, C. Deng, P. Yang, X. Zhang, Proteomics 8 (2008) 2778–2784.
[16] H. Chen, D. Qi, C. Deng, P. Yang, X. Zhang, Proteomics 9 (2009) 380–387.
[17] W. Jia, X. Chen, H. Lu, P. Yang, Angew. Chem. Int. Ed. 118 (2006) 3423–3427.
[18] Y. Zhang, X. Wang, W. Shan, B. Wu, H. Fan, X. Yu, Y. Tang, P. Yang, Angew. Chem. Int. Ed. 117 (2005) 621–623.
[19] H. Kawasaki, T. Yonezawa, T. Watanabe, R. Arakawa, J. Phys. Chem. C 111 (2007) 16278–16283.
[20] X. Wen, S. Dagan, V.H. Wysocki, Anal. Chem. 79 (2007) 434–444.
[21] W.W. Shen, H.M. Xiong, Y. Xu, S.J. Cai, H.J. Lu, P. Yang, Anal. Chem. 80 (2008) 6758–6763.

[22] E.T. Castellana, D.H. Russell, Nano Lett. 7 (2007) 3023–3025.
[23] K. Shrivas, S.K. Kailasa, H.F. Wu, Proteomics 9 (2009) 2656–2667.
[24] A. Suryajaya, F. Nabok, A. Davis, S.P.J. Hassan, J. Higson, Appl. Surf. Sci. 254 (2008) 4891–4898.
[25] A.D. Yoffe, Adv. Phys. 51 (2002) 799–890.
[26] L. Pellegrino, A. Tirelli, Int. Dairy J. 10 (2000) 435–442.
[27] T.B. Ng, S.K. Lam, S.Y. Chan, Peptides 23 (2002) 1361–1365.
[28] I. Hannus, Gy. Tasi, I. Kiricsi, J.B. Nagy, H. Forster, P. Fejes, Thermochim. Acta 249 (1995) 285–294.
[29] P.R. Sudhir, H.F. Wu, Z.C. Zhou, Anal. Chem. 77 (2005) 7380–7385.
[30] J. Zhou, W. Ens, K.G. Standing, A. Verentchikov, Rapid Commun. Mass Spectrom. 6 (1992) 671–678.
[31] R.C. Beavis, B.T. Chait, NATO ASI Series, Series B: Phys. 269 (1991) 227–234.
[32] S. Kjellstrom, O.N. Jensen, Anal. Chem. 76 (2004) 5109–5117.
[33] Z. Liu, K.L. Schey, J. Am. Soc. Mass Spectrom. 19 (2008) 231–238.
[34] L. Fedele, R. Seraglia, B. Battistotti, C. Pinelli, P. Traldi, J. Mass Spectrom. 34 (1999) 1338–1345.
[35] P.C. Lin, M.C. Tseng, A.K. Su, Y.J. Chen, C.C. Lin, Anal. Chem. 79 (2007) 3401–3408.