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RESEARCH ARTICLE

Ga_2O_3 photocatalyzed on-line tagging of cysteine to facilitate peptide mass fingerprinting

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β-Ga_2O_3 is a wide-band-gap semiconductor having strong oxidation ability under light irradiation. Herein, the steel target plates modified with β-Ga_2O_3 nanoparticles have been developed to carry out in-source photo-catalytic oxidative reactions for online peptide tagging during laser desorption/ionization mass spectrometry (LDI-MS) analysis. Under UV laser irradiation, β-Ga_2O_3 can catalyze the photo-oxidation of 2-methoxyhydroquinone added to a sample mixture to 2-methoxy benzoquinone that can further react with the thiol groups of cysteine residues by Michael addition reaction. The tagging process leads to appearance of pairs of peaks with an *m/z* shift of 138.1Th. This online labelling strategy is demonstrated to be sensitive and efficient with a detection-limit at femtomole level. Using the strategy, the information on cysteine content in peptides can be obtained together with peptide mass, therefore constraining the database searching for an advanced identification of cysteine-containing proteins from protein mixtures. The current peptide online tagging method can be important for specific analysis of cysteine-containing proteins especially the low-abundant ones that cannot be completely isolated from other high-abundant non-cysteine-proteins.

Keywords:
Database / MS / Peptide mass fingerprinting / Surface modification / Technology

1 Introduction

Protein profiling is one of the most challenging areas in bioanalytical science, limited by the enormous complexity of protein samples [1]. Analytical strategies with high sensitivity and resolution are required to address the issue. The most common approach for analyzing complex protein samples is mass spectrometry (MS)-based protein characterization flow [2], combining the techniques of sample extraction/separation, high-resolution mass spectrometer and bio-informatics analysis often based on spectra query against sequence databases. Despite many recent progresses, MS-based proteomics still faces significant technical challenges, such as the increasing demand for higher resolution to improve identification accuracy, especially when dealing with a very large number of proteins present in a single organism [3]. Specifically, when MS is used to identify the proteins separated by 2-D PAGE or HPLC [4], it is common to find several proteins in one separated fraction due to the limited resolution of 2-D PAGE or HPLC, therefore making difficult the MS-based protein identification. To date, a common way to circumvent this problem is to recur to high-resolution measurements using high-cost mass spectrometers, such as Orbitrap [5] and Fourier-transform ion cyclotron resonance [6].

An alternative approach is to gather supplementary information in addition to peptide masses to constrain database search [7]. Such additional information can be the isoelectric point (p*I*) of peptides or the content of one

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Abbreviations: LDI-MS, laser desorption/ionization mass spectrometry; **MOHQ,** 2-methoxyhydroquinone; **MOWSE,** molecular weight search; **TEM,** transmission electron micrographs; **XRD,** X-ray powder diffraction

Colour Online: See the article online to view Scheme 1, Figs. 4 and 5 in colour.

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specific amino acid residue within peptides. Based on the principle, some strategies have been developed, such as OFFGEL electrophoresis for detecting p*I* of peptides [8] and peptide-labeling methods for counting the contents of a specific amino acid residue in peptides [7, 9–14]. In the former strategy, peptide mixtures are separated into fractions according to their isoelectric points, whereas in the latter strategy, specific amino acids are tagged and then sent to MS for analysis. By comparing the m/z shift between tagged and untagged peaks of the same peptide, the information on the number of a given amino acid in a peptide can be obtained. Of course for the strategy to be efficient, the target amino acids need to be well chosen [15]. Cysteine, which is contained in 89.3% of all proteins in humans [16] and ranks among the most frequently found in protein functional sites [17–19], is thereby a good candidate.

Owing to the ability of thiols to undergo redox reactions, cysteine is one of the most chemically active amino acid and shows antioxidant properties for cell detoxification [20]. The chemical tagging of free cysteines has been well developed with the purpose of protein quantification [21, 22], and mapping of protein functional sites [17–19]. The offline chemical reaction on cysteins often requires a time-consuming sample preparation procedure. Accordingly, designing strategies for online and high-throughput analysis is a persistent challenge. Electrochemical tagging methods have been reported for online labelling cysteine residues using a microchip electrospray ionization (ESI) source [7, 11, 12, 23]. A microspray device with an integrated carbon electrode was used to electrochemically generate species able to react specifically with cysteine moieties in an acidic spray condition to realize peptide tagging, summarized in a recent review [24].

As another important soft ionization technique in addition to ESI, matrix-assisted laser desorption ionization (MALDI) is also widely used. Various modifications have been introduced on the target plate of MALDI-MS instrument to realize specific functions [14, 25]. When the plate is modified with photosensitive materials, complex photochemical redox reactions can take place within nanoseconds in the MALDI ion source after laser irradiation [26–28]. We have designed before TiO_2-modified target plate to carry out laser desorption ionization (LDI) in-source photochemical redox reactions for disulfide bond cleavage, peptide tagging and peptide *a,x*-fragmentation [14, 25, 29].

Herein, we developed a new photo-sensitive substrate by sintering β-Ga_2O_3 sol–gel [30] on a steel plate for realizing efficient online cysteine tagging during MALDI. Because of its wide-band gap of approximate 4.8 eV [31, 32], β-Ga_2O_3 has emerged as an exceptional catalytic or supporting material that is highly efficient for a broad range of acid-catalyzed reactions, such as dehydrogenation [33, 34]. Under laser irradiation during LDI, electrons can be excited from the valence band to the conduction band of Ga_2O_3 nanocrystals, yielding oxidative holes and reductive electrons, which can further react with the present samples on the plate [35]. 2-methoxyhydroquinone (MOHQ) is selected here as a target molecule for photo-oxidation due to the high electronic density in its phenyl rather than other substituted hydroquinones. The MOHQ can be photo-oxidized to 2-methoxybenzoquinone by the valence band holes, and the latter can further form complexes with cysteine residues in peptides by Michael addition. It was indeed reported that the photo-generated electron–hole pairs of Ga_2O_3 have stronger oxidative capability than those of TiO_2 [36]. Therefore, the labelling strategy here is very efficient and sensitive being able to selectively tag the cysteine-containing peptides from tryptic digests of protein mixtures, and further promoting the specific identification of cysteine-containing proteins from protein-mixtures without pre-separation.

2 Materials and methods

2.1 Materials

Peptides SSDQFRPDDCT (C-pep) (90%, M_w 1269.5) and ACKCTCM (3C-pep, MW 758.3) were obtained from Shanghai HD Biosciences. Diammonium citrate (99%) was purchased from Amresco. ACN (99.9%) and TFA (99.8%) were purchased from Merck (Darmstadt, Germany), while ammonium bicarbonate (99%), MOHQ (98%), gallium nitrate, CHCA (99%), β-lactoglobulin A (from bovine milk, 90%), BSA (Bos Taurus, 95%), myoglobin (from horse heart, 95%) and trypsin (from bovine pancreas) were obtained from Sigma (St. Louis, MO, USA). All these reagents were used as received without further purification. Deionized water (18.2 MΩ cm) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2 Preparation and characterization of nanocrystalline β-Ga_2O_3

An alcoholic gel-precipitation pathway was employed to synthesize the nanocrystalline β-Ga_2O_3 [30]. In a typical process, concentrated aqueous ammonia and ethanol (50:50 in volume) was added dropwise to the ethanol solution of gallium nitrate hydrate until no more precipitation occurred. The resulting gel was quickly filtered and washed by ethanol, dried at 100°C overnight, and finally calcinated at 600°C for 5 h.

The X-ray powder diffraction (XRD) of β-Ga_2O_3 was performed on a Bruker D8 Advance X-ray diffractometer using nickel filtered Cu Ka radiation at 40 kV and 20 mA. Transmission electron micrographs (TEM) were recorded digitally using a Gatan slow-scan charge-coupled device camera on a JEOL 2011 electron microscope operating at 200 kV. Samples for electron microscopy were prepared by grinding and dispersing the powders in ethanol, and by applying a drop of the very dilute suspension on carbon-coated grids.

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2.3 Modifying β-Ga_2O_3 array on a target plate

Fabrication of β-Ga_2O_3-modified target plate was performed using a sintering strategy [14]. The β-Ga_2O_3 was diluted in deionized water to obtain a stable suspension with a material concentration of 1 mg/mL, then dropped on a stainless steel plate as an array of spots (~2 μL each) and dried at room atmosphere and temperature overnight. The modified plate was then heated in an oven at 400°C for 1 h, naturally cooled-down to room temperature and stored at 60°C in an oven.

2.4 Preparation of protein digestions

Nearly, 1 mg β-lactoglobulin A, BSA or myoglobin was dissolved in 1 mL ammonium bicarbonate (25 mM, pH ~8), denatured at 100°C for 5 min and digested for 12 h at 37°C with an enzyme to protein ratio of 1:30 (w/w). After proteolysis, 2 mM DTT was added into the digest of β-lactoglobulin A or BSA, and the mixture was incubated at 37°C for 1 h to break the disulfide bonds. The reduced samples were used immediately for subsequent tagging experiments to avoid the cysteine auto-oxidation.

2.5 MS detection and data analysis

Nearly, 0.4 μL mixture of peptide/MOHQ or protein-digests/MOHQ was deposited on the β-Ga_2O_3 array immediately after mixing and dried at room temperature and atmosphere in dark for ~10 min. After drying, ~0.4 μL of CHCA matrix (5 mg/mL in 50% ACN/49.9% H_2O/0.1% TFA) was dropped and dried in the same conditions. Finally the sample was subjected to MALDI-TOF-MS analysis (Applied Biosystems 4700 Proteomics Analyzer, 2000 laser shots (355 nm, 200 Hz) with a laser intensity of 5500 instrument units). For comparison, ~0.4 μL of peptide, peptide/MOHQ mixture or protein digests was dropped on a normal target plate, dried in the same conditions, followed with the deposition of ~0.4 μL CHCA matrix and MALDI-TOF-MS analysis (Applied Biosystems 4700 Proteomics Analyzer, 2000 laser shots (355 nm, 200 Hz) at a laser intensity of 5500 instrument units).

MS data analysis was performed with the Data Explorer™ Software from Applied Biosystems and the MASCOT search engine available on the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) (http://expasy.org/). The masses and intensities of special peaks were read out using the Data Explorer™ Software. Cysteine-containing peptides were identified through finding a pair of peaks with an m/z shift of 138.1Th directly from one mass spectrum. The entire list of observed peaks (with $S/N > 3$, except the peaks of tagged peptides) were submitted to MASCOT sequence query to make a search in the Swiss-Prot database with or without giving information about the cysteine content. The cysteine content information is specified for cysteine-containing peptides by "comp(number[C])" entered after the peptide m/z. The m/z tolerance was set as ± 0.5. The maximum miss cleavage sites for tryptic digestion was set as 3.

3 Results and discussion

3.1 Characterization of β-Ga_2O_3 photo-sensitive target plate

To analyze the oxidation products of a molecule by MS, a two-step approach is usually employed: the molecule is first oxidized either chemically using strong oxidants or electrochemically on an anode or even photo-chemically under light irradiation, and then the resulting products are analyzed by MS. The present investigation aims at operating these procedures in a single step by incorporating directly samples together with a photosensitizer on a MALDI target plate, where photo-oxidative reactions can occur upon light irradiation during sample ionization processes. Accordingly, a photosensitive β-Ga_2O_3-modified target plate has been designed to work as a photo-electrode during MALDI with the application of the voltage for ion extraction. Scheme 1 (right) schematically represents the β-Ga_2O_3-assisted in-source cysteine peptide tagging. β-Ga_2O_3 is a wide-band-gap semiconductor consisting of separated valence band and conduction band. A YAG laser at 355 nm was used for MALDI, and electrons were excited from the valence band to conduction band of β-Ga_2O_3 by a two-photon process as indicated by the red arrow in Scheme 1. The photo-excitation left oxidative holes on the valence band, which could oxidize the reductive probe molecule, MOHQ, to 2-methoxybenzoquinone (MOBQ) that further reacted with cysteine-containing peptides to form complexes through the Michael addition reaction. After the in-source reactions, all the products could then be released in gas phase as ions and accelerated by electric field for MS analysis. The overall reaction is likely to be very complex because the oxidation and tagging reactions should happen in nanoseconds following the sample ionization induced by the laser shot. As a result, in addition to the final products, many intermediate active species, such as radicals, can also be generated. Here, it is likely that the oxidation is initiated on the surface or in the nanopores of the β-Ga_2O_3 structure; while the further tagging and proton transfer processes occur in the plume generated after the laser shot.

The nanocrystalline β-Ga_2O_3 support was prepared by an alcoholic gel-precipitation method [30]. The XRD pattern of the as synthesized nanocrystalline support shows well-defined diffraction features characteristic of β-Ga_2O_3 (Fig. 1B). TEM shows that the support is highly porous in nature, consisting of interconnected particles with average crystallite size of 7–8 nm (Fig. 1A). This porous structure

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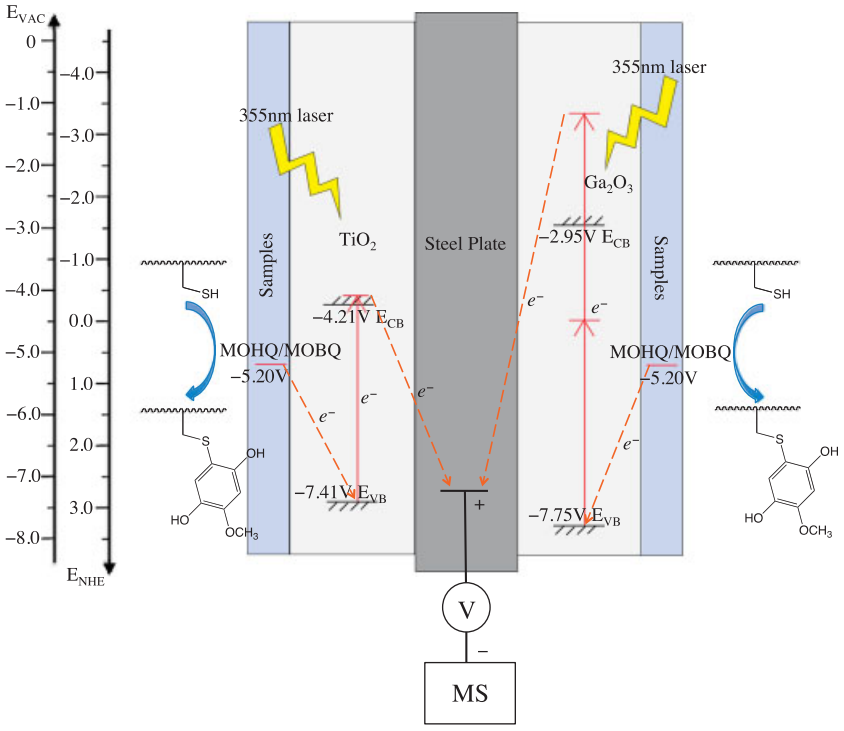

Scheme 1. Schematic illustration of the tagging of cysteine-containing peptides catalyzed by β-Ga_2O_3 or TiO_2 under laser irradiation. E_{CB}: conduction band edge energy. E_{VB}: valence band edge energy. E_{NHE}: normal hydrogen electrode energy. E_{VAC}: absolute vacuum energy. The intrinsic energy levels of the two semiconductors listed in the scheme are respect to absolute vacuum energy referring to a publication from [32]. The oxidation potential of MOHQ refers to a former publication from our group [23]

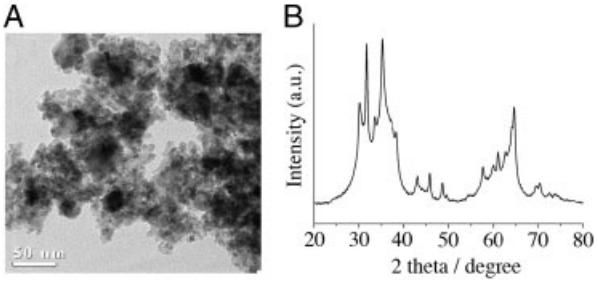

Figure 1. (A) TEM image and (B) XRD pattern of β-Ga_2O_3.

results in a very high specific surface area of ~84 m^2/g. Considering that the tagging reaction should happen either on the surface or in the nanopores of the β-Ga_2O_3 nanocrystalline layer, the large specific surface area would lead to a very efficient in-source reaction. Furthermore, compared with TiO_2 nanoparticles that we have employed before for inducing LDI in-source sample oxidation [14], Ga_2O_3 should in principle show stronger oxidation ability under the irradiation of light. As shown in Scheme 1, the valence band edge energy of Ga_2O_3 is lower than that of TiO_2, resulting in a bigger energy drop when electrons transfer from MOHQ to the valence band of excited Ga_2O_3. Indeed, Wu et al. compared systematically the oxidation of salicylic acid by TiO_2 (P25, Degussa) and various β-Ga_2O_3 nanoparticles, and found that the β-Ga_2O_3 nanoparticles obtained under solvothermal conditions of alcohols, which are quite similar as what we employed here, hold much stronger oxidation ability than TiO_2 [36].

3.2 In-source tagging of cysteine-containing peptide

To verify that the present β-Ga_2O_3-modified MALDI target plate can be employed to realize efficient in-source tagging reaction, a peptide containing one cysteine residue, SSDQFRPDDCT (C-pep, MW 1269.5), was employed as the target sample. Experiments were performed either on the β-Ga_2O_3-modified plate or a normal target plate, where a mixture of C-pep and MOHQ (1:1 in mol) was deposited for subsequent MS analysis. The resulting mass spectrum obtained on the β-Ga_2O_3-photo-electrode plate (Fig. 2B) exhibited two peaks at 1408.6 and 1270.5, which correspond to the protonated forms of MOHQ-tagged and untagged C-pep, respectively. In contrast, only one peak (1270.5) was observed on the mass spectrum obtained from the unmodified target plate (Fig. 2A), illustrating that the photo-oxidation of MOHQ on the as-prepared photosensitive β-Ga_2O_3 spots resulted in a cysteinyl tagging during LDI-MS measurement. With this strategy, the presence of a cysteine residue in a peptide can be clearly detected. As shown on the mass spectrum of Fig. 2B, the tagged species showed a peak about 20 × stronger than that of the untagged one, indicating a very high tagging efficiency associated with the photocatalysis of the nanocrystalline β-Ga_2O_3. This online tagging method is also vey sensitive. Indeed, as shown in Fig. 2C, the tagging peak could still be clearly observed even when the sample concentration was lowered to 4 fmol, reaching the instrument detection limit. Additionally, when a peptide containing several cysteines was utilized, i.e. ACKCTCM (3C-pep, MW 758.3), the signals from singly, doubly, and triply tagged peptides could all

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be observed on one spectrum together with the untagged peptides, indicating the presence of three cysteines in the peptide, Fig. 2D.

With these results, it is clear that the β-Ga_2O_3 can induce efficient oxidation reaction under light irradiation by a multi-photon process. Since the electron would be excited to a very high energy state after absorbing two photons, large amount of energies could be released when the electrons in the conduction band of β-Ga_2O_3 migrate into the steel plate driven by the applied voltage during the ion extraction process of MALDI, thereby further promoting the overall photo-oxidation reaction.

3.3 Online peptide tagging for selectively enhanced identification of cysteine-containing proteins

The high tagging efficiency makes the photo-electrochemical labeling method promising to be applied in selectively analyzing cysteine-containing peptides in protein digests. To demonstrate this principle, three proteins, myoglobin, β-lactoglobulin A and BSA, were employed. After digestion and disulfide bond reduction, the obtained tryptic peptides were deposited together with MOHQ on the β-Ga_2O_3 photo-electrode and analyzed by MALDI-MS. By finding pairs of peaks with an *m/z* shift of 138.1Th directly from the obtained mass spectra, the cysteine content information was obtained. The entire list of observed peaks (with $S/N > 3$, except the peaks of tagged peptides) was submitted to MASCOT sequence query to make a search in the Swiss-Prot database with or without giving information about the cysteine content.

β-Lactoglobulin A is a protein containing five cysteine residues [14]. Using the in-source peptide tagging strategy, three peptides were tagged, all corresponding to single-cysteine-containing peptides (Table 1, Fig. 3A). Without giving information about the cysteine content, β-lactoglobulin A was identified with a molecular weight search (MOWSE) score of 85 by the MASCOT sequence query, Fig. 4A. When the information about cysteine content of the tagged peptides was given, the MOWSE score was raised to 92, Fig. 4B.

Owing to the limited resolution of current separation methods, it is very common that several proteins may be

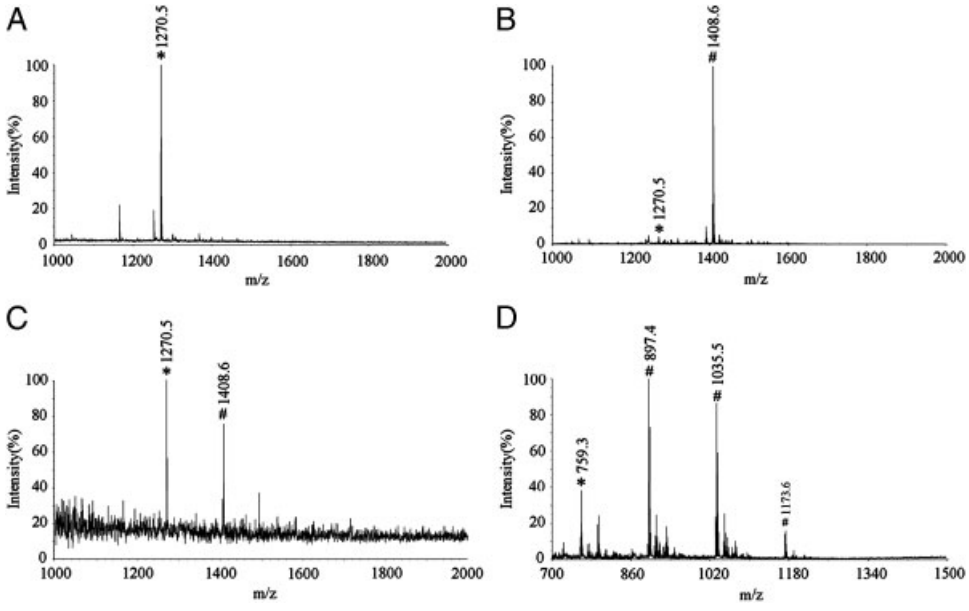

Figure 2. Mass spectra of C-pep/MOHQ (1:1, 300 fmol each) deposited (A) on a blank target plate and (B) on a β-Ga_2O_3-modified target plate, respectively, with CHCA matrix; (C) Mass spectrum of C-pep/MOHQ (1:1, 4 fmol each) deposited on a β-Ga_2O_3-modified target plate with CHCA matrix; and (D) Mass spectrum of 3C-pep/MOHQ (1:1) deposited on a β-Ga_2O_3-modified target plate with CHCA matrix. ∗: protonated native peptides; ♯: protonated tagged peptides.

Table 1. Identified cysteine-containing peptides from β-lactoglobulin A digest and a mixture of myoglobin digest and β-lactoglobulin A digest

m/z of the observed precursor and tagged peptides[#]	Number of cysteine	Sequence of precursor peptides	β-Lactoglobulin digest	Digest of myoglobin and β-lactoglobulin mixture
1122.5, 1260.6[#]	1	WENDECAQK	√	√
1250.6, 1388.7[#]	1	WENDECAQKK	√	√
1658.8, 1796.9[#]	1	LSFNPTLQEEQCHI	√	√

All protein concentrations were fixed at 0.8 μM
√: observable on mass spectra.

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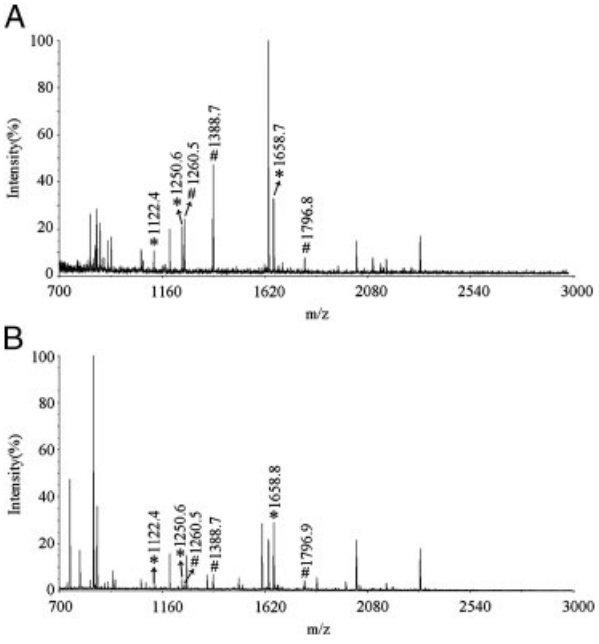

Figure 3. Mass spectra of (A) β-lactoglobulin A digest (320 fmol) and (B) the mixture of β-lactoglobulin A digest (320 fmol) and myoglobin digest (320 fmol) in the presence of 10ng/μL MOHQ (0.4 μL) obtained on a β-Ga_2O_3-modified target plate with CHCA matrix. *: protonated native peptides; #: protonated tagged peptides.

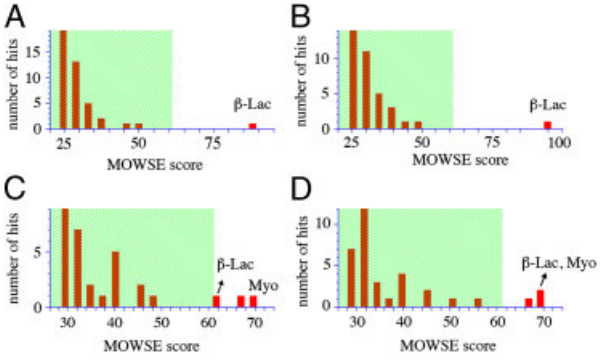

Figure 4. MASCOT processing results of a mass spectrum of β-lactoglobulin A digest (320 fmol) (A) without and (B) with considering the cysteine content information, and a mass spectrum of a mixture of β-lactoglobulin digest (320 fmol) and myoglobin digest (320 fmol) (C) without and (D) with considering the cysteine content information. The concentration of MOHQ was fixed at 10 ng/μL. Identifications in green area are not believable.

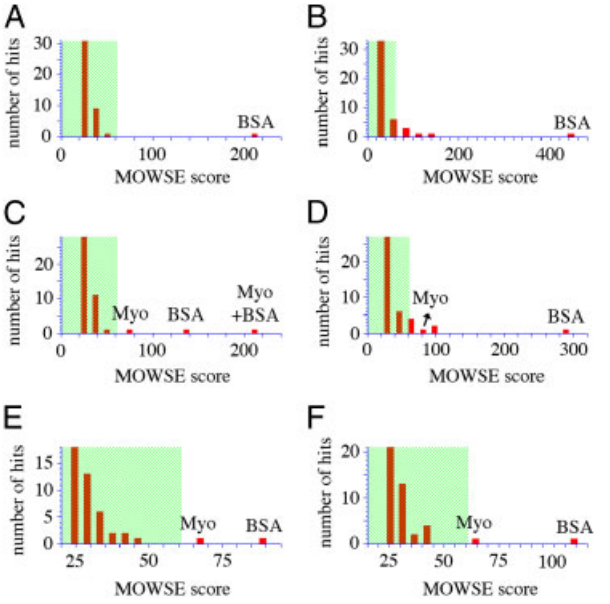

Figure 5. MASCOT processing results of a mass spectrum of BSA digest (320 fmol) (A) without and (B) with considering the cysteine content information, a mass spectrum of a mixture of BSA digest (320 fmol) and myoglobin digest (320 fmol) (C) without and (D) with considering the cysteine content information, and a mass spectra of a mixture of BSA digest (320 fmol) and myoglobin digest (3.2pmol) (E) without and (F) with considering the cysteine content information. The concentration of MOHQ was fixed at 10 ng/μL. Identifications in green area are not believable.

presented in one faction from HPLC or one spot on the 2-D PAGE, which are then digested and deposited onto one spot of a MALDI target plate for MS analysis, therefore resulting in poor identification because of the difficulty during database query.

Here, we prove that the identification of cysteine-containing proteins from a protein mixture can be selectively enhanced using the in-source tagging strategy. A tryptic digest mixture of myoglobin (containing no cysteine) and β-lactoglobulin A with the ratio of 1:1 in mol was utilized to illustrate this application. As shown in Table 1 and Fig. 3B, the three single-cysteine-containing peptides digested from β-lactoglobulin A were still tagged and identified. Without giving the information about cysteine content, β-lactoglobulin A was identified from the mixture with a MOWSE score of only 62, which just passed the limitation for successful identification, while myoglobin was identified with a MOWSE score of 70 (Fig. 4C). In contrast, by adding the information about cysteine content, both β-lactoglobulin A and myoglobin were identified with a score of 69 (Fig. 4D), showing that the identification accuracy of β-lactoglobulin A was enhanced.

In the case of β-lactoglobulin A, the identification enhancement was not very prominent because there are only few cysteine residues on β-lactoglobulin A. It can be expected that the selectively enhanced identification would be more effective when a protein with many cysteine residues are employed, such as bovine serum albumin, a protein containing 35 cysteine residues [37, 38]. Using the in-source peptide tagging strategy, 21 peptides digested from BSA were tagged, including 13 single-cysteine-containing peptides, 6 two-cysteine-containing peptides,

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Table 2. Identified cysteine-containing peptides from BSA digest and a mixture of myoglobin digest and BSA digest with different mole ratios

m/z of observed precursor and tagged peptides[#]	Number of cysteine	Sequence of precursor peptides	BSA digest	Digest mixture of myoglobin and BSA (1:1)	Digest mixture of myoglobin and BSA (10:1)
701.5, 839.6[#]	1	GACLLPK	√	√	×
841.5, 979.6[#]	1	LCVLHEK	√	√	×
1011.4, 1149.5[#]	1	QNCDQFEK	√	√	×
1024.5, 1162.6[#], 1300.7[#]	2	CCTESLVNR	√	√	×
1050.5, 1188.6[#]	1	EACFAVEGPK	√	√	×
1052.5, 1190.6[#], 1328.7[#]	2	CCTKPESER	√	×	×
1177.6, 1315.7[#], 1453.8[#]	2	ECCDKPLLEK	√	×	×
1349.6, 1487.7[#], 1625.8[#]	2	TCVADESHAGCEK	√	×	×
1362.7, 1500.8[#]	1	SLHTLFGDELCK	√	√	√
1386.6, 1524.7[#]	1	YICDNQDTISSK	√	√	√
1418.7, 1556.8[#], 1694.9[#]	2	LKECCDKPLLEK	√	√	×
1519.8, 1657.9[#]	1	LKPDPNTLCDEFK	√	√	√
1578.7, 1716.8[#], 1854.9[#], 1993.0[#]	3	ECCHGDLLECADDR	√	√	×
1616.8, 1754.9[#]	1	QEPERNECFLSHK	√	√	×
1633.7, 1771.8[#], 1909.9[#]	2	YNGVFQECCQAEDK	√	×	×
1667.8, 1805.9[#]	1	MPCTEDYLSLILNR	√	×	√
1823.9, 1962.0[#]	1	RPCFSALTPDETYVPK	√	√	√
1963.1, 2101.2[#]	1	LKPDPNTLCDEFKADEK	√	√	×
2076.9, 2215.0[#], 2353.1[#], 2491.2[#]	3	ECCHGDLLECADDRADLAK	√	√	×
2091.0, 2229.1[#]	1	LKPDPNTLCDEFKADEKK	√	√	×
2484.2, 2622.3[#]	1	QEPERNECFLSHKDDSPDLPK	√	√	×

BSA concentration was fixed at 0.8 μM
√: observable on mass spectra; ×: unobservable on mass spectra.

and 2 three-cysteine-containing peptides (Table 2). By considering the abundant cysteine content information, the identification score could be increased from 221 to 445 as shown in Fig. 5A and B. When a mixture of myoglobin and BSA (1:1 in mol) was employed, 16 peptides of the tryptic digest mixture of the two proteins could still be tagged, as shown in Table 2. Without considering the cysteine content information, BSA was identified from the mixture with a MOWSE score of only 132, while 289 of MOWSE score was obtained for BSA from the same mixture by adding the information of cysteine content during database query, Fig. 5C and D. Benefiting from the highly efficient tagging and the large number of cysteine residues, the present specifically enhanced identification of BSA can be further realized with the presence of excessive non-cysteine proteins. By employing the mixture of myoglobin and BSA at the ratio of 10:1 in mol as an example, five peptides digested from BSA were successfully tagged, Table 2. The identification score of BSA in the mixture could then be increased from 89 to 109 by considering the cysteine content information, whereas the MOWSE score for myoglobin stayed at 66, indicating that the identification of BSA from excessive non-cysteine containing proteins was selectively enhanced, Fig. 5E and F. It should be mentioned that the decrease in the number of labeled peptides should be mainly a result of the poor ionization rather than the tagging reaction. In the presence of a large amount of myoglobin digests, the peaks of myoglobin digests dominated the mass spectra, and therefore only few very strong peaks of BSA digests still existed on the spectra. Indeed, by comparing the database searching results of mass spectra obtained on the β-Ga_2O_3 photo-electrode and a normal target plate, it was found that all the cysteine-containing peptides observed were successfully tagged.

4 Concluding remarks

In summary, we have developed a nanocrystalline β-Ga_2O_3-derived target plate to realize efficient in-source photocatalytic oxidations for specific peptide tagging during LDI. Using this β-Ga_2O_3 photo-electrode plate, the information of cysteine contents and peptide masses can be obtained simultaneously during a single experiment. Protein mixtures were employed to demonstrate the feasibility and selectivity for the facile analysis of cysteine-containing proteins. Further extensive investigation of specific online peptide tagging using the β-Ga_2O_3-derived photo-sensitive plate on more representative samples coupled with 2-D-gel

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will facilitate practical application of the MALDI in-source redox reactive approach in enhancing MS protein mapping.

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