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De novo sequencing of tryptic peptides sulfonated by 4-sulfophenyl isothiocyanate for unambiguous protein identification using post-source decay matrix-assisted laser desorption/ionization mass spectrometry

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A simple method of solid-phase derivatization and sequencing of tryptic peptides has been developed for rapid and unambiguous identification of spots on two-dimensional gels using post-source decay (PSD) matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The proteolytic digests of proteins are chemically modified by 4-sulfophenyl isothiocyanate. The derivatization reaction introduces a negative sulfonic acid group at the N-terminus of a peptide, which can increase the efficiency of PSD fragmentation and enable the selective detection of only a single series of fragment ions (y-ions). This chemically assisted method avoids the limitation of high background normally observed in MALDI-PSD spectra, and makes the spectra easier to interpret and facilitates *de novo* sequencing of internal fragment. The modification reaction is conducted in C_{18} μZipTips to decrease the background and to enhance the signal/noise. Derivatization procedures were optimized for MALDI-PSD to increase the structural information and to obtain a complete peptide sequence even in critical cases. The MALDI-PSD mass spectra of two model peptides and their sulfonated derivatives are compared. For some proteins unambiguous identification could be achieved by MALDI-PSD sequencing of derivatized peptides obtained from in-gel digests of phosphorylase B and proteins of hepatic satellite cells (HSC).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) is the most important tool for protein identification by analysis of protein digests. Due to its very short analysis time, its high sensitivity and ease of automation, MALDI-peptide mass fingerprinting (PMF) has become the preferred method for identifying proteins for which the sequences are available in databases.[1–11] Unfortunately, PMF can also lead to ambiguous protein identification because of insufficient protein sequence coverage, complex mixtures of tryptic peptides from co-migrating proteins, and novel proteins with no matches in the databases.[12,13] Consequently, only ~50% of the proteins excised from gels can be identified by PMF. To alleviate this problem, additional information reflecting the primary sequence of the investigated proteins is required for their unambiguous identification.

Recently, post-source decay (PSD) MALDI-MS was developed for high-sensitivity peptide sequencing applications due to its advantages in requiring a less demanding purification, as well as its efficiency and ability to identify amino acid modifications.[13,14] Even so, several problems are also associated with PSD-MALDI sequencing of peptides, including the poor signal/noise (S/N) ratio of the observed fragment ions, incomplete coverage of the peptide sequence, the complexity of the resulting fragmentation patterns, and the lack of computer algorithms capable of interpreting the more complex spectra.[13,15] The poor S/N and incomplete sequence coverage may be attributed to the inadequate internal energy deposition in PSD, and the complexity of the spectra can be explained by formation of many types of fragment ions. During the desorption/ionization or collision activation, energy is deposited in the protonated peptides to effect the cleavage of bonds all along the peptide backbone. The site of protonation directs fragmentation to yield the amino acid sequence specific ions. Although protonation preferentially occurs at the N-terminal amino group or the amino groups of basic residues, in activated ions the charge can be randomly localized on the nitrogen atom of any one of the amide bonds and on the carbonyl oxygen atoms. Thus, any of the peptide bonds can be broken in several different ways.

Several different chemical labeling approaches have been developed to enhance the S/N ratio of fragment ions, to increase the coverage of the peptide sequence, and to eliminate the difficulties in interpreting the complex spectra obtained by PSD-MALDI-MS. Charge derivatization of

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peptides, involving the attachment of a fixed positive or negative charge to one terminus, has been developed by a number of laboratories over the past decade.[12–15] The use of a variety of charged groups, including a quaternary ammonium group, phosphonium acetyl group, acetylated sulfhydryl group, aromatic sulfonic acid group and chlorosulfonyl group, has been investigated.[15–18] The viability of charge derivatization methods depends on the efficiency of the derivatization process, as well as the success in producing a limited range of predictable sequence-specific fragment ions. Many charged derivatives have been developed; however, few are routinely used. This may be because of low yields and harsh derivatization conditions; for example, a non-aqueous environment is required during labeling by sulfobenzoic acid cyclic anhydride or by chlorosulfonylacetyl chloride.

The introduction of an N-terminal sulfonic acid group was applied to successfully enhance fragmentation towards the peptide–amide bond (CO-NH) by Keough and co-workers.[12,15] This reaction is very similar to Edman degradation, and the present work can be regarded as an extension of the work of Keough *et al*. Phenyl isothiocyanate (PITC, Edman reagent) is a perfect chemical for amino acid sequencing, so we had reason to believe that 4-sulfophenyl isothiocyanate (SPITC) would also be well suited to derivatization and fragmentation of peptides in MALDI-PSD sequencing. The work of Gevaert and co-workers verified the feasibility of this theory.[19] However, their work leaves two problems to be solved. First, the detection of peptides carrying a fixed negative charge in a background of residual unmodified or doubly modified (peptides containing lysine residues) peptides in positive ion mode in MALDI-MS is difficult and complex, and lowers the overall sensitivity. Second, due to the requirement of pre-separation by RP-HPLC, the initial amount of protein material needed for this type of sequencing has to be rather high (nmol amounts are needed), leading to significant difficulties in the sequencing of peptides from a 2D-gel digest in proteome analysis. Marekov and co-worker identified a Coomassie-stained protein band from a 1D gel of human CLSP using SPITC.[20] Due to the low protein levels encountered in proteomics research, the higher sensitivity silver staining method is used in most laboratories. In this paper we demonstrate an improved solid-phase derivatization method, including guanidination and sulfonation of tryptic peptides from silver-stained 2D gels, by *O*-methylisourea hydrogen sulfate and SPITC, respectively, for high sensitivity *de novo* sequencing at sub-picomole levels in proteomics research.

EXPERIMENTAL

Chemicals

4-Sulfophenyl isothiocyanate (SPITC) was obtained from Aldrich (Cat. no. 85,782-3), and rabbit phosphoryrase b, bovine serum albumin (BSA), TPCK-trypsin and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Sigma (St. Louis, MO, USA). *O*-Methylisourea hydrogen sulfate, lysine-terminated model peptide (HGTVVLTALGGILK, Mr 1378) and arginine-terminated model peptide (ADSGEGDFLAEGGGVR, Mr 1536) were obtained from Amersham Biosciences (Uppsala, Sweden). C_{18} μZipTips™ were purchased from Millipore (Bedford, MA, USA). All other chemicals were analytical reagent grade and used without further purification.

Separation and in-gel digestion of proteins

Proteins were separated according to the description of Liang *et al*.[21] Two-dimensional electrophoresis was performed using the IPG-SDS-PAGE system (Amersham Biosciences, Uppsala, Sweden). The first-dimension separation was performed on a 13-cm linear Immobiline strip (pH range 3–10). The second-dimension separation run was performed on 9–15% polyacrylamide linear gels (13 cm × 20 cm × 1.0 mm). The gel was then stained with silver to visualize protein spots.

Protein bands were destained with 30 mM $K_3Fe(CN)_6$/100 mM $Na_2S_2O_3$ 1:1 (v/v) for 2–3 min and then washed with water. The bands were excised, crushed into pieces, and enough acetonitrile was added to dehydrate the gel particle before drying in a vacuum centrifuge. The gel particles were then allowed to swell in 10 mM dithiothreitol/100 mM NH_4HCO_3 and incubated for 45 min at 57°C to reduce the protein. The liquid was replaced with the same volume of freshly prepared 55 mM iodoacetamide/100 mM NH_4HCO_3 solution, and was then incubated for 30 min at room temperature in the dark. The gel particles were washed with 100 mM NH_4HCO_3 and completely dried in a Speed Vac, and then were immersed in digestion buffer (50 mM NH_4HCO_3, 5 mM $CaCl_2$, 0.1 mg/L of trypsin) at 37°C overnight.

Sample binding to the ZipTip

All derivatization procedures were performed on solid-phase supports, C_{18} μZipTips. A ZipTip was wetted with 50% acetonitrile/0.5% trifluoroacetic acid (TFA) by slowly aspirating and dispensing the solution up and down a few times, then equilibrated with 0.1% TFA. The sample to be derivatized was dissolved in 0.1% TFA, and drawn up and down the ZipTip about 10 times to absorb onto the reversed-phase material. After a wash with 0.1% TFA, the sample was ready for *in situ* derivatization.

Modification of Lys-terminated peptide by *O*-methylisourea

The reaction was carried out according to the description given by Keough *et al*.[12] A freshly prepared solution of *O*-methylisourea hydrogen sulfate (0.5 M in 0.25 M Na_2CO_3, pH 11.7) was allowed to react with the bound sample by leaving the reagent in the tip for 2 h at 37°C in order to convert the ε-amino groups on lysine residues to guanidyl groups. Excess reagent was washed out using 0.1% TFA.

N-Terminal derivatization of peptides with SPITC

4-Sulfophenyl isothiocyanate was prepared at a concentration of 2.55 mg/mL in Tris-HCl buffer (50 mM, pH 8.2). This solution was aspirated into contact with the bound sample on the ZipTip (making sure no bubbles were present), then left in the ZipTip and incubated at 50°C. After 1 h, excess reagent was washed out with 0.1% TFA and the derivatized peptide(s) were eluted in 2.5 μL of 80% acetonitrile/0.5% TFA for MALDI analysis.

Mass spectrometry

All mass spectrometry experiments were performed using an Applied Biosystems (Framingham, MA 01701, USA) Voyager DE-STR time-of-flight mass spectrometer equipped with a N_2 laser (337 nm, 3 ns pulse width, 20 Hz repetition rate). All mass spectra were acquired in the reflectron mode with delayed extraction. External mass calibration was performed with a mixture of peptide standards (angiotensin I, *m/z* 1296.6853; ACTH fragment 1–17, *m/z* 2093.0867; ACTH fragment 18–39, *m/z* 2465.1989), and mass-measurement accuracy was typically ±0.3 Da.

PSD fragment ion spectra were acquired for peptides and peptide derivatives after isolation of the appropriate precursor ions by using timed ion selection. Fragment ions were refocused onto the final detector by stepping the voltage applied to the reflectron in the following ratios: 1.000 (precursor ion segment), 0.918, 0.830, 0.777, 0.650, 0.483, 0.316, 0.178 and 0.106 (fragment ion segments). Care was taken to adjust the laser power so that unit mass resolution was obtained, and 200–300 laser shots were collected for each voltage segment. The individual segments were stitched together using software developed by Applied Biosystems. The PSD fragment ions were measured as isotopically averaged masses.

The matrix used for all mass spectra was α-cyano-4-hydroxycinnamic acid (CHCA), as a saturated solution in 1% TFA in acetonitrile/water (1:1, v/v). Samples for MS were prepared by using 2 μL of the charge-derivatized peptide solution mixed with 5 μL of the matrix solution, and applied to the stainless-steel sample plate. The mixture was air dried before insertion into the mass spectrometer.

De novo sequencing of peptides from a protein digest

The *de novo* sequencing of peptides from a tryptic digestion was performed as follows. Approximately 10% of protein digest was analyzed by PMF, and if a significant identity was found, further action was often not necessary. However, if no reliable match was obtained, the remaining 90% of the digest was derivatized as described. The derivative was first analyzed in normal MALDI reflector mode, and *m/z* values resulting from addition of either 215 or 257 Da to the *m/z* values observed in the original spectrum were subjected to PSD analysis. Even a low precursor ion signal was usually enough to generate a clean PSD spectrum, thus allowing sequencing of all derivatized peptides visible in the mass spectrum. The presence of neighboring peptides was considered when setting a window around the target mass.

Database searching

The PSD mass spectra were searched in two ways against the NCBI non-redundant protein sequence database. First, uninterpreted PSD spectra were searched with the MS-Tag program from the Protein Prospector suite of search tools developed at UCSF. Search inputs included the measured precursor and fragment ion masses. The conservative error tolerances typically used were ±0.5 Da for the monoisotopic precursor ion and ±1.0 Da for the isotopically averaged fragment ions. Only y-type fragment ions were allowed possibilities. Other types of fragment ions, such as a, b, (b+H_2O), (b+NH_3), and internal cleavages, were not considered. Alternatively, the PSD data were manually interpreted. The derived sequence tags were searched using the MS-Edman program from the Protein Prospector software package. MS-Edman does not require the precursor or fragment ion masses as inputs, but only uses the measured sequence tags. The program considers all combinations of ambiguous residues, like (K,Q and E) or (I, N and D), which have similar masses.

RESULTS AND DISCUSSION

Derivatization and PSD sequencing of a model peptide

The present solid-phase derivatization worked well with two model peptides, a lysine-terminated peptide (Lys-peptide, HGTVVLTALGGILK $[M+H]^+$ at *m/z* 1378.8422) and an arginine-terminated peptide (Arg-peptide, ADSGEGDFLAEGGGVR $[M+H]^+$ at *m/z* 1536.6930). A recent publication[22] suggests that the intensity of the detected ions is biased toward ions with an arginine residue over those with a lysine residue, and that was indeed the case in our experiments. As shown in Fig. 1, the intensity of the

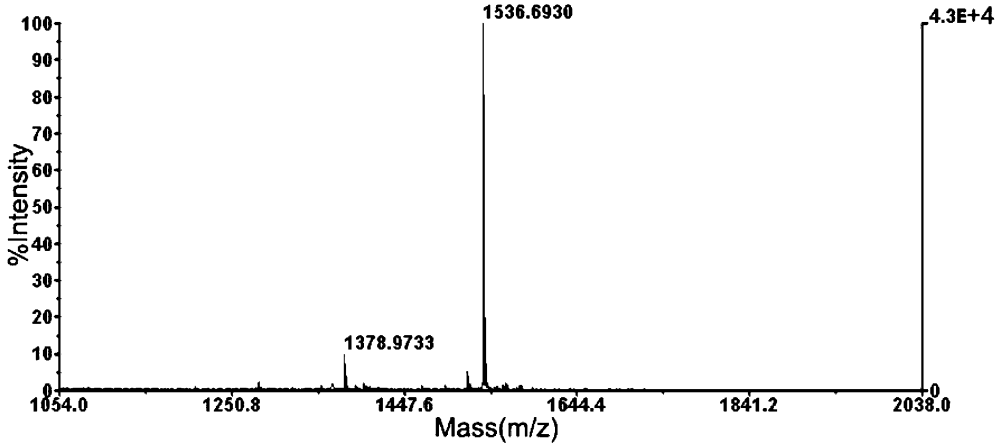

Figure 1. MALDI mass spectrum of an equimolar mixture of two model peptides, a lysine-terminated (HGTVVLTALGGILK, MH^+ at *m/z* 1378.8422) and a arginine-terminated (ADSGEGDFLAEGGGVR, MH^+ at *m/z* 1536.6930) peptide. The arginine-terminated peptide is detected with about 10-fold greater intensity than the lysine-terminated peptide.

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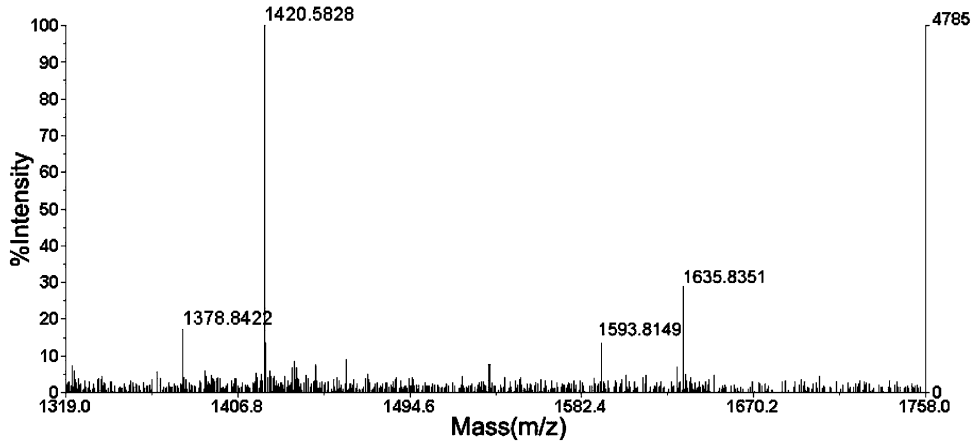

Figure 2. MALDI mass spectrum of a mixture containing the intact lysine-terminated peptide (MH^+ at *m/z* 1378.84), the guanidinated lysine-terminated peptide (MH^+ at *m/z* 1420.58), the sulfonated lysine-terminated peptide (MH^+ at *m/z* 1593.81), and the sulfonated homoarginine-terminated peptide (MH^+ at *m/z* 1635.83).

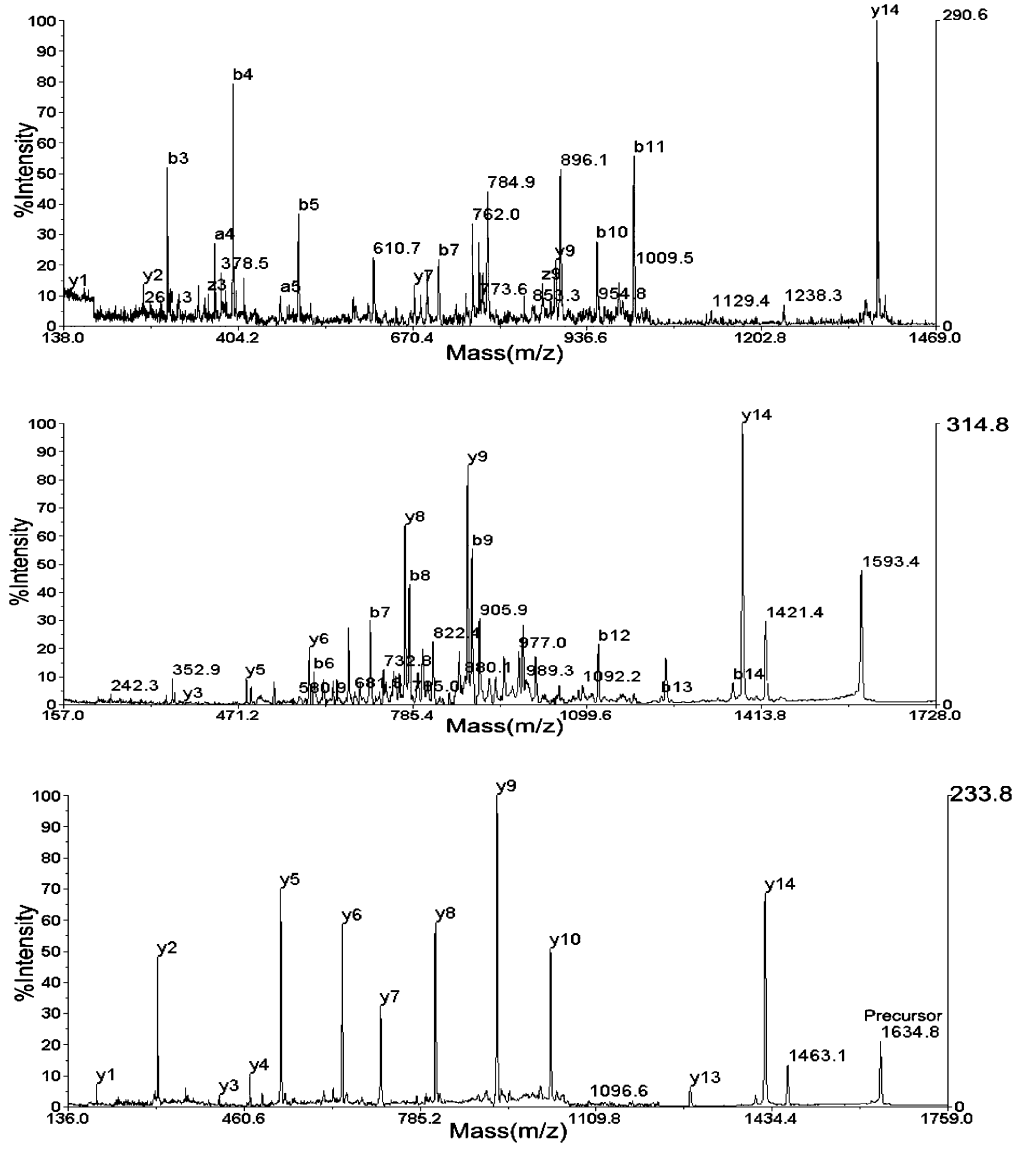

Figure 3. PSD-MALDI spectra obtained from the lysine-terminated peptide HGTVVLTALG-GILK. Intact peptide (top), sulfonated peptide (middle), and sulfonated homoarginine-terminated peptide (bottom). The spectra show the influence of the various chemical modifications on the peptide fragmentation patterns.

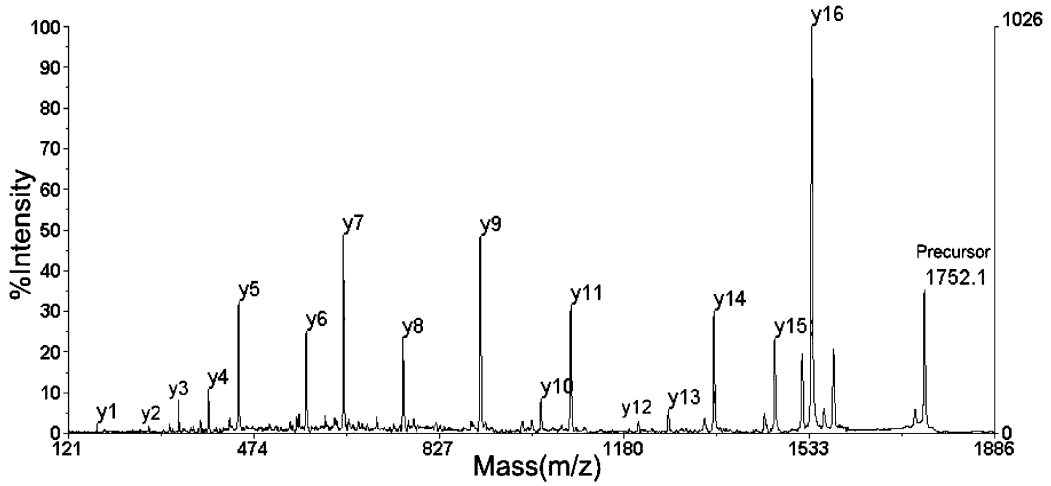

Figure 4. The PSD-MALDI mass spectrum obtained from the sulfonated arginine-terminated model peptide (ADSGEGDFLAEGGGVR, MH^+ at *m/z* 1752.1).

prominent peak at *m/z* 1536.6930, due to Arg-peptide, is approximately 10-fold higher than that of Lys-peptide at *m/z* 1378.8422. Guanidination of the ε-amino group of lysine side chains is a simple and useful way to solve the sensitivity problem associated with lysine-terminated tryptic peptides. Salts of *O*-methylisourea have been shown to specifically react with the ε-amino group of lysine side chains even in the presence of free primary amines at the N-termini of tryptic peptides.[22–26] Therefore, guanidination was introduced into our experiments for two purposes, namely, to convert lysine residues to homoarginines to enhance the intensities of lysine-terminated peptide ions in MALDI-MS, and to prevent unwanted sulfonation of the ε-amino groups of lysine side chains to avoid double modification of lysine-terminated peptides which would lead to a sensitivity decrease in MALDI-MS; thus conversion to homoarginines left the free N-termini of these peptides available for sulfonation prior to PSD sequencing.

Derivatization of our test Lys-peptide was obtained by derivatizing the ε-amino group of the C-terminal lysine and then sulfonating the N-terminus. Figure 2 shows MALDI mass spectrum of the mixture of the intact peptide and of the guanidinated Lys-peptide modified by 4-sulfophenyl isothiocyanate (SPITC). After guanidination by *O*-methylisourea, the MALDI intensity of Lys-peptide was obviously enhanced, and the guanidination product was shifted to *m/z* 1420.8640 (Fig. 2), reflecting the 42 Da mass increment resulting from addition of a single guanidinium group to the molecule. However, in the case of the guanidiated peptide further modified by SPITC, the intensity of the peak decreases (Fig. 2, the modified peptide is shifted to *m/z* 1635.8351 as a result of adding both the guanidinium (42 Da) and phenyl sulfonate groups (215 Da) to the native peptide). This may be attributed to the fact that the negative charge of the added sulfonic acid groups neutralizes the positive charge of the protonated guanidinium groups. The precursor ion selector was set at *m/z* 1378.84, 1593.81 and 1635.84, and both derivatized and underivatized peptides were subjected to PSD sequencing. The PSD spectra of intact Lys-peptide, Lys-peptide modified by SPITC and

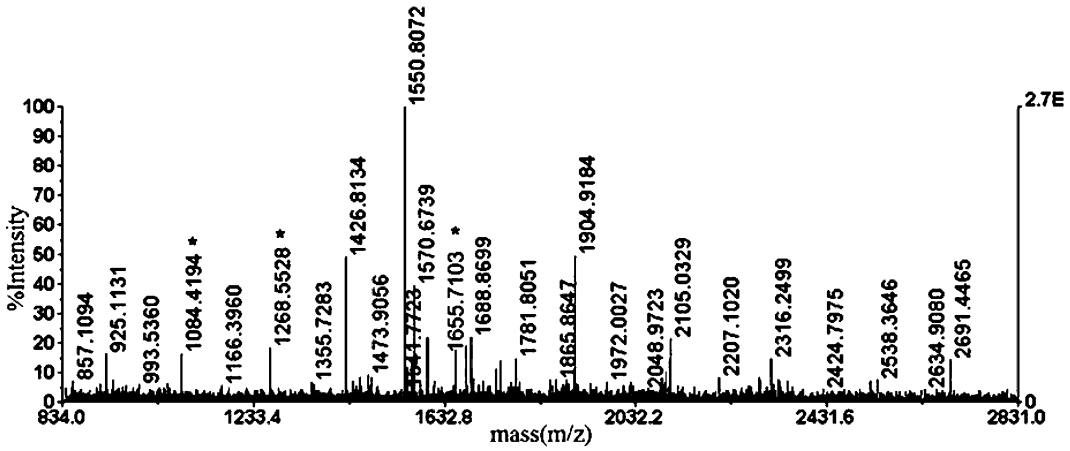

Figure 5. MALDI mass spectrum of the derivatized digest of rabbit phosphorylase b, excised from a 15% SDS-PAGE gel, in-gel digested with trypsin and modified with SPITC. The peaks marked with their respective monoisotopic *m/z* values indicate those peptides that were sulfonated. The three candidates for PSD sequence analysis are marked with an asterisk.

guianidinated Lys-peptide modified by SPITC are compared in Fig. 3. The spectrum obtained from the intact Lys-peptide demonstrates a complex fragmentation pattern consisting of incomplete series of N-terminal a- and b-type ions, C-terminal y- and z-ions, and even internal cleavage products. The spectrum would be difficult to accurately interpret *de novo*. The spectrum from the sulfonated Lys-peptide, in which mainly b- and y-type ions appear, makes the interpretation easier than for the unmodified peptide but unfortunately some unexpected fragments are still present in the spectrum. This method, in which a lysine-terminated peptide was modified directly by SPITC without guanidination protection, is not ideal for *de novo* sequencing.

Figure 3 also shows the spectrum obtained by analysis of the sulfonated guanidinated peptide. This sulfonated homoarginine derivative produces mainly y-type fragment ions with excellent S/N ratios and clear background under MALDI-PSD conditions. The spectrum can be readily interpreted *de novo*. This result may be attributed to introduction of a negative charge (sulfonic acid group) at the N-terminus of the peptide. After derivatization, the formation of a positively charged ion (net charge) would require two protons to be introduced into the peptide. One of these protons will primarily reside in the basic C-terminal side chain but the other has a higher degree of freedom to mobilize along the peptide backbone, assisting fragmentation to mainly b- and y-fragments. However, in view of the negative charge (sulfonic acid group) at the N-terminus, the b-fragments are overall neutral resulting in a spectrum in which only the y-ions are observed.

The MALDI-PSD mass spectrum obtained from a sulfonated arginine-terminated model peptide is shown in Fig. 4.

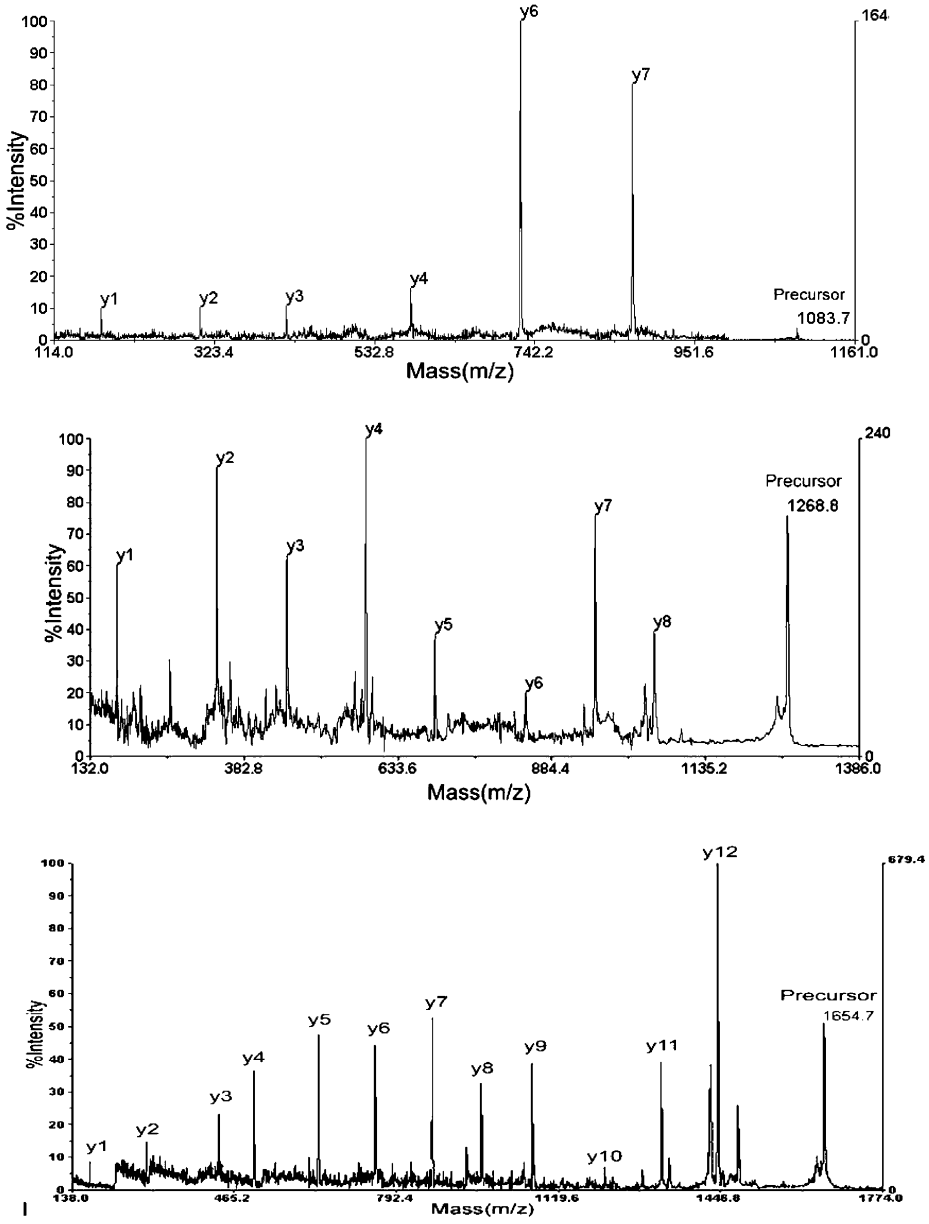

Figure 6. MALDI-PSD analysis of three selected sulfonated peptides from the rabbit phosphorylase b in-gel digest. PSD-MALDI spectrum of the peptide of *m/z* 1084.42 (top), m/z 1268.55(middle), and m/z 1655.71(bottom). These three precursor ions were indicated with asterisks in Fig. 5. All three mass spectra provided definitive identification of the parent protein.

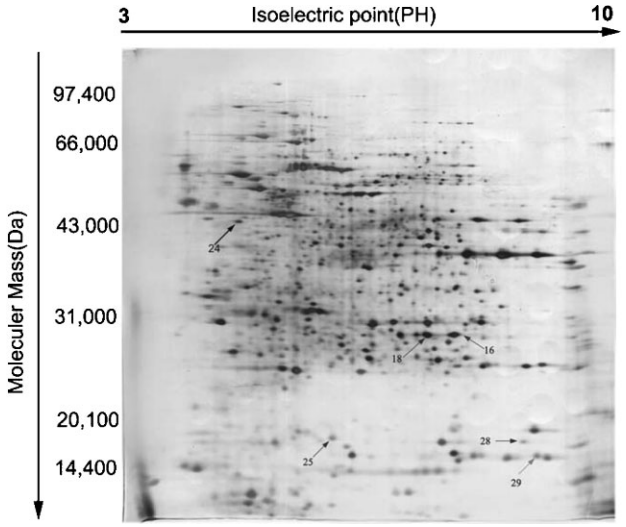

Figure 7. Protein map of the mouse hepatic satellite cell (HSC) treated by a Chinese herbal medicine compound prescription. The protein spots identified by PSD sequencing are indicated with arrows.

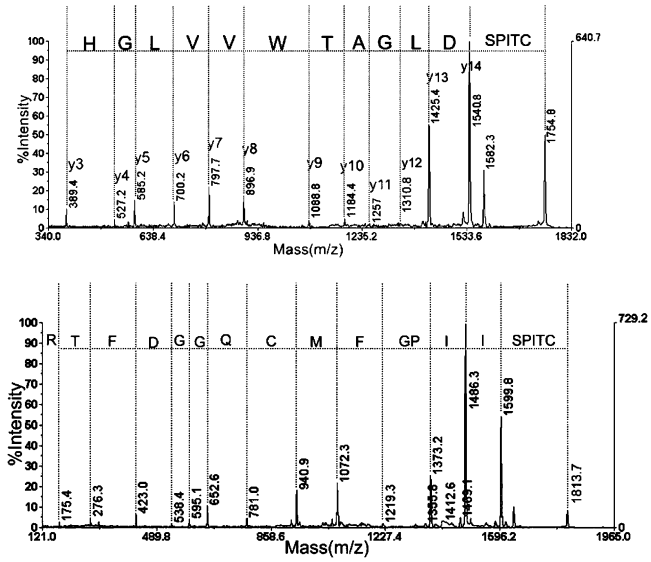

Figure 8. MALDI-PSD analysis of two selected sulfonated peptides from the in-gel digests of two different HSC proteins. PSD-MALDI spectra of the fragment (DLGATWVVLGHSER) from HSC-18 in-gel digest (top), and of the fragment (IIPGFMCQGGDFTR) from HSC-29 in-gel digest (bottom).

The fragment ion peaks of derivatized Arg-peptide represent the complete y1 to y16 y-ion series plus the labeled precursor ion (+215 Da), making the amino acid sequence easily interpretable by manual calculation of the differences between the adjacent y-ion fragments, or by using suitable simple software. As compared with lysine-terminated peptides, arginine-terminated peptides are more suitable for *de novo* PSD-MALDI sequencing due to the simple one-step derivatizion procedure, excellent S/N ratios, and unambiguous interpretation.

Derivatization and PSD sequencing of proteins from in-gel digests

Some standard proteins from an SDS-PAGE gel, including rabbit phosphorylase b, bovine serum albumin, and rabbit actin, were subjected to in-gel digestion, sulfonation, and PSD sequencing. The MALDI mass spectrum obtained from the sulfonated derivative of the rabbit phosphorylase b digest is shown in Fig. 5. By comparison with the corresponding spectrum of the non-sulfonated digest (not shown), it was possible to identify the sulfonated peptides (Fig. 5) including MH^+ ions at *m/z* 1084.40 (817–823), 1268.53 (643–650), 1570.67 (51–61), 1595.63 (761–771), 1665.71 (522–533), 1688.83 (388–399), 1904.88 (193–206), 2104.99 (372–387), and 2207.05 (440–458). Figure 6 shows three examples of PSD sequencing of the peptides at *m/z* 1084.40, 1268.53, and 1655.71, all of which yielded some of the lower-abundance signals among the sulfonated peptides (Fig. 5). All PSD spectra show mainly complete y-ion series with excellent S/N. Some small peaks still can be found in the spectra, but these are of much lower abundance than the y-ions and do not confuse the sequence interpretation of the target peptide. They all provided unambiguous protein identification, as phosphorylase was the only candidate protein when searched against the MS-Tag program (Protein Prospector). This result indicates that low-abundance precursor ions can give a sufficiently strong signal and generate a fully readable PSD spectrum.

We recently used these solid-phase sulfonation and PSD sequencing methods as part of a proteomics project that is being conducted on hepatic satellite cells (HSC). After induction by EB virus, a mouse liver cell line was incubated with a Chinese herbal medicine compound prescription (named as '861') for 2 days. We tried to find differently expressed proteins. All the proteins from silver-stained 2D gels were subjected to in-gel digestion and PMF identification (about one-tenth of a tryptic digest loaded into the mass spectrometer). Ambiguous proteins were subjected to guanidination and sulfonation on solid-phase supports (C_{18} μZipTips) for subsequent PSD sequencing. Figure 8 shows the PSD-MALDI mass spectra obtained from two different protein spots (Fig. 7, HSC-18, 29). The derivatized peptide with *m/z* 1754.8 from the HSC-18 spot yielded y-type fragment ions at *m/z* 391.0, 528.0, 585.2, 698.2, 797.7, 896.9, 1082.9, 1185.1, 1255.6, 1310.8, 1425.4, and 1540.8 (Fig. 8, top), which is well matched with the y3 to y14 sequence of DLGATWVVLGHSER (precursor ion *m/z* 1539.79) derived from the protein triosephosphate isomerase (TPI). The derivatized peptide at *m/z* 1813.7 from the HSC-29 spot produced y-type fragment ions at *m/z* 175.4, 276.4, 422.8, 538.4, 594.9, 652.6, 780.8, 940.6, 1071.9, 1220.1, 1372.5, 1486.6, and 1600.0 (Fig. 8, bottom) which is well matched with the y1 to y10 and y12 to y14 sequence ions of IIPGFMCQGGDFTR (precursor ion *m/z* 1598.74) derived from the protein cyclophilin (peptidylprolyl isomerase). In both of these examples unambiguous protein identification was obtained when searched against the entire NCBI protein sequence database (MS-Tag program from Protein Prospector).

Some other protein spots of HSC have been unambiguously identified by this solid-phase derivatization and PSD sequencing method. Their protein names, molecular masses, pI values, and matched sequences are shown in Table 1. These proteins include pr22 gene product, heat shock 73 protein, triosephosphate isomerase, and cyclophilin

Table 1. The expressed proteins of HSC identified by sulfonation and PSD sequencing methods

Spot number*	Sequence	Protein name	MW/pI
16	FFVGGNWK	Triosephosphate isomerse	26712/6.9
18	DLGATWVVLGHSER	Triosephosphate isomerase	26696/6.9
24	ARFEELNADLFR	Heat shock 73 protein	70871/5.4
25	ASGQAFELILSPR	Pr22 gene product	17275/5.8
29	IIPGFMCQGGDFTR	Cyclophilin (peptidylprolyl isomerase)	17971/7.7

*Spot numbers refer to Fig. 7.

(peptidylprolyl isomerase). In these experiments some proteins gave PSD spectra with excellent quality, but there was no protein sequence match from the database. It is possible that the sequence of that particular protein is not yet available.

CONCLUSIONS

The solid-phase guanidination and sulfanation procedures for in-gel tryptic digests outlined here provide a powerful method for high-sensitivity *de novo* peptide sequencing using MALDI-PSD. The guanidination reaction protects the ε-amino groups against side reactions and increases the utility of the derivatives for Lys-terminated tryptic peptides. The labeling with a sulfonic acid group by 4-sulfophenyl isothiocyanate (SPITC) enhances PSD sequencing of Arg- and Lys-terminated peptides, producing mainly y-type fragment ions with excellent signal-to-noise ratios and clear background under MALDI-PSD conditions, and the spectra can be readily interpreted *de novo*. Thus the amino acid sequence can be simply calculated from the mass differences between the adjacent y-ions, and advanced interpretation software is not required. The combination of peptide mass fingerprinting (PMF) and PSD sequencing described here could become a useful method for unambiguous protein identification in proteome studies.

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