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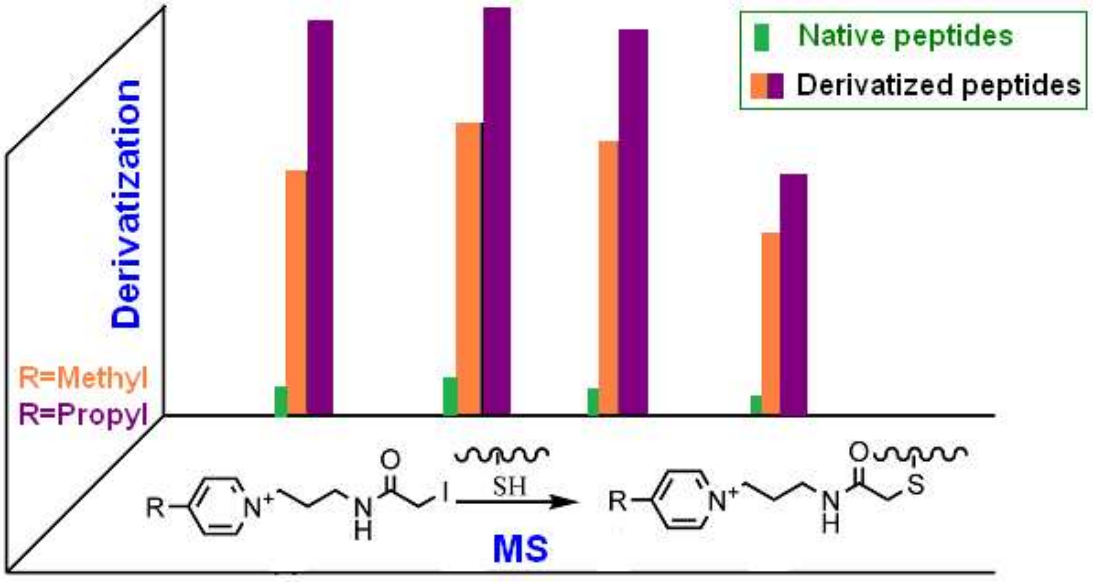
Graphical Abstract



Novel pyridinium-based tags were exploietd for highly efficient analysis of thiol-containing peptides by mass spectrometry

Novel pyridinium-based tags: Synthesis and characterization for highly efficient analysis of thiol-containing peptides by mass spectrometry

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In this study, novel type of pyridinium-based tags, 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-4-methylpyridinium bromide (IMP) and 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-4-propylpyridinium bromide (IPP), were designed, synthesized, and further exploited for derivatization of thiol-containing peptides. With model peptides as the sample, the labeling efficiency and the stability of peptide derivative were investigated. The results indicated that nearly 100% derivatization yield was achieved with the developed tags and the peptide derivative could stabilize at room temperature for at least one week. Furthermore, improved ionization efficiency and increased charge states were achieved via both matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) and electrospray ionization (ESI) MS, of which IPP exhibited the more obvious improvement of ionization efficiency. Further analysis of tryptic digests of bovine serum albumin (BSA) and α-transferrin, increased identification efficiency of thiol-containing peptides was achieved by combination with IMP or IPP derivatization. For example, the identification efficiency of the thiol-containing peptides of α-transferrin increased more than 42% by combination with IMP or IPP derivatization. We anticipate the novel tags will be promising for high efficient thiol-containing peptide identification in proteome research, especially for those with low concentration.

1. Introduction

Since the completion of human genome sequencing, proteome research has been paid much attention. One of the challenges of proteome research is the tremendous dynamic range and high complexity of real samples [1]. Thus, many proteins and peptides are difficult to be detected, especially for those with low abundance [2]. In fact, many of them, such as glycoproteins and phosphoproteins, often play key role in the human body, and even some of them have been proved to be related to various cancer treatment or diagnosis [3,4]. Therefore, it is very important to develop sensitive detection techniques for efficient analysis of these samples.

Because of the superior sensitivity and excellent merit to provide structural information, mass spectrometry (MS) has become the first choice for both qualitative and quantitative analysis of proteins/peptides in proteome research [5,6]. However, the detection sensitivity of proteins/peptides, especially for those with low concentration and poor ionization efficiency, still can hardly meet the requirements of real sample analysis [7,8]. Chemical derivatization is a widely used technique for improving analysis and detection of these proteins and peptides. Recently, many MS-based tags, such as imidazolium derivatives [9-11], piperazine derivatives [12-15], guanidine derivatives [16,17], alkyl tertiary amino or quaternary ammonium derivatives [18-23], phenyl derivatives [24-26], and pyridine derivatives [27-30], have been exploited for derivatization of various reactive groups on the peptides with improved ionization efficiency and enhanced detection sensitivity. These tags were systematically summarized in our recent reported review [31].

As a rare amino acid in protein samples, cysteine specific tags [10,11,18,19,23-25,32] have been paid much more attention and widely exploited to improve analysis of thiol-containing peptide by MS. For example, Qiao et al. developed a new kind of imidazolium-based tags and further exploited for thiol-containing peptides derivatization, of which

1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-3-hexylimidazolium bromide (IPHI), possessing strongest hydrophobicity, could largely improve the ionization efficiency of peptides via both matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS and electrospray ionization (ESI) MS. Moreover, the average charge states of the derivatized peptides could also be increased [10,11]. Zabet-Moghaddam et al. reported using N-ethyl maleimide and iodoacetanilide to derivatize the thiol groups on peptides. The number of identified peptides from protein bovine serum albumin (BSA) could be increased to 33 from 24 via MALDI-TOF MS and MASCOT search engine while the score of the protein could be increased to 255 from 203, achieving high confident identification [25]. Shuford et al. evaluated two commercially available tags and several newly synthesized hydrophobic tags, such as 2-iodo-N-octylacetamide, 2-iodo-N-dodecylacetamide, 2-iodo-N-benzylacetamide, 2-iodo-N-(phenethyl)acetamide, 2-iodo-N-(4-phenylbutyl)acetamide, and N-(6-(2-iodoacetamido)hexyl)benzamide. The detection limit of B-type Natriuretic peptide, a low abundance cardiac biomarker, could be decreased about 3.5-fold via 2-iodo-N-octylacetamide derivatization and ESI MS analysis [32].

N-substituted pyridinium salts are a kind of ionic liquid using in various sub-disciplines of analytical chemistry, such as enantioseparation [33,34], pharmaceutical analysis [35], and biochemical analysis [36]. Herein, to the best of our knowledge, two novel pyridinium-based tags, including 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-4-methylpyridinium bromide (IMP) and 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-4-propylpyridinium bromide (IPP), were firstly designed, synthesized, and further exploited for derivatization of thiol groups on peptides, of which the tag IPP indicated the most obvious ionization efficiency increment via both MALDI-TOF MS and ESI MS.

2. Experimental

2.1. Chemicals and Reagents

4-Methylpyridine was purchased from J&K Scientific (Beijing, China). 4-Propylpyridine, iodoacetic acid, iodoacetamide (IAA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), BSA and α-transferrin from bovine were obtained from Sigma-Aldrich (St. Louis, MO, USA). 3-Bromopropylamine hydrobromide was from Aladdin Reagent (Shanghai, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl) and peptides with the sequences CDPGYIGSR, LEACTFRRP, MECFG, and ALVCEQEAR were purchased from GL Biochem (Shanghai, China). α-Cyano-4-hydroxycinnamic acid (CHCA) was obtained from Bruker Daltonics (Bremen, Germany). Trifluoroacetic acid (TFA) was from Acros Organics (Geel, Belgium). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q system (Millipore, Molsheim, France).

2.2. Synthesis of IMP

1-(3-Aminopropyl)-4-methylpyridinium bromide was firstly synthesized. In brief, 3-bromopropylamine hydrobromide (0.9 g, 4 mmol) and 4-methylpyridine (1.0 g, 10 mmol) were dissolved in 5 mL of ethanol. After the above mixture was refluxed for 24 h under nitrogen atmosphere, solvent ethanol was removed and the oil residues were repeatedly washed with diethyl ether in order to remove excess 4-methylpyridine, dried again to yield 1-(3-aminopropyl)-4-methylpyridinium bromide.

For IMP, iodoacetic acid (14 mg, 0.08 mmol) and the synthesized 1-(3-aminopropyl)-3-methylpyridinium bromide (20 mg, 0.08 mmol) were sequentially added into 0.4 mL of acetonitrile/water (1:1, v:v) solvent at 0 °C, followed by vortexing for roughly 10 s. Then, EDC•HCl (18 mg, 0.09 mmol) was added, and the resulting mixture was further stirred at 0 °C for 1 h. Finally, the product was purified by C18-RP column using acetonitrile-water as the eluent. NMR spectra were determined on a Bruker AVANCE III 600 MHz spectrometer, and high-resolution (HR) MS spectra were determined on a Bruker apex

ultra 7.0 T Fourier transform mass spectrometer (Bruker, Bremen, Germany). ^{1}H NMR (600 MHz, DMSO): δ=8.90 (d, J=6.7 Hz, 2H), 8.36 (s, 1H), 8.00 (d, J=6.3 Hz, 2H), 4.53 (t, J = 6.6 Hz, 2H), 3.17 (s, 2H), 3.01-3.16 (m, 2H), 2.62 (s, 3H), 2.14-2.02 (m, 2H). HRMS, m/z: 319.03005 (Calculated: 319.03073).

2.3. Synthesis of IPP

The synthesis procedure of IPP was similar to that of IMP. Firstly, 1-(3-aminopropyl)-3-propylpyridinium bromide was synthesized by refluxing of 3-bromopropylamine hydrobromide and 4-propylpyridine for 24 h under nitrogen atmosphere. Then, the synthesized product 1-(3-aminopropyl)-3-propylpyridinium bromide was reacted with iodoacetic acid with the presence of EDC•HCl to yield the final product IPP. ^{1}H NMR (600 MHz, DMSO): δ=9.01 (d, J=6.6 Hz, 2H), 8.62 (s, 1H), 8.03 (d, J=6.4 Hz, 2H), 4.58 (t, J=7.1 Hz, 2H), 4.09 (s, 2H), 2.91-2.84 (m, 2H), 2.60-2.64 (m, 2H), 2.38-2.41 (m, 2H), 1.66-1.74 (m, 2H), 0.93 (t, J=7.3 Hz, 3H). HRMS, m/z: 347.06097 (Calculated: 347.06203).

2.4. Prediction of gas-phase hydrogenation capacity and Log P value

The gas-phase hydrogenation capacity of the tags IMP and IPP was calculated based on the software ORCA 2.8 (Website: http://www.thch.uni-bonn.de/tc/orca/) with the method B3LYP/TZVP [37-39]. The Log P values representing the hydrophobicity of the tags were predicted based on the on-line software Molinspiration (Website: http://www.molinspiration.com/cgi-bin/properties).

2.5. Peptide derivatization

The derivatization procedure for the model peptides was as follows. An aliquot of 5 μL of peptides CDPGYIGSR, LEACTFRRP, ALVCEQEAR with the concentration of 1 mg/mL or MECFG with the concentration of 0.5 mg/mL were firstly reduced at 56 °C for 1 h by the addition of 10 μL of 20 mM TCEP in 50 mM Tris-HCl buffer (pH~8.4). Then, 30 μL of 50

mM IMP or IPP were added to derivatize the thiol groups on the peptides by further incubation at 37 °C for 1 h in the dark. The derivatized peptides could be directly used for MS determination. The derivatization procedure of these peptides via IAA was the same as that derivatized by IMP or IPP, except that IMP or IPP was replaced by IAA.

2.6. Protein derivatization and digestion

Proteins BSA and α-transferrin were firstly denatured via urea. Then, an aliquot of 5 μg of BSA or α-transferrin was reduced by the addition of 10 μL of 20 mM TCEP for 1 h at 56 °C. The thiol groups of the proteins were derivatized by the addition of 30 μL of 50 mM IMP or IPP for another 1 h at 37 °C in dark. After the proteins were diluted until the concentration of urea lower than 1.0 M, trypsin was added to allow the ratio of protein to enzyme of 20:1. The proteins were digested at 37 °C overnight, desalted and redissolved in 550 μL of water, ready for further MS analysis. The derivatization procedure of the proteins via IAA was the same as that derivatized by IMP or IPP, except that IMP or IPP was replaced by IAA.

2.7. MS analysis

MALDI mass spectra were acquired by a Bruker Ultraflex III TOF/TOF mass spectrometer instrument (Bruker, Bremen, Germany). The peptide solution (1 μL) was firstly deposited on the polished steel target. After it dried, matrix solution (1 μL, 7 mg/mL CHCA in 0.1% TFA/60% acetonitrile) was deposited. The identification of the proteins was based on the peptide mass fingerprints (PMF) to search the database of Mammalia via MASCOT server (Matrix Science, London, UK). The parameters were set as follow: enzyme, trypsin; max missed cleavages, 2; peptide mass tolerance, ±200 ppm; fixed modifications, carbamidomethyl (C) via IAA derivatization or modifying cysteine residue with 190.1106 Da or 218.1419 Da via IMP or IPP derivatization, respectively.

ESI mass spectra were determined on a LTQ Orbitrap XL mass spectrometer (Thermo

Finnigan, San Jose, CA, USA).

3. Results and discussion

3.1. Characteristics of the developed tags

Gas-phase basicity and hydrophobicity of the analytes are important factors for their ionization by MS [40,41]. Thus, pyridinium-based tags, IMP and IPP, designed with strong gas-phase basicity and high hydrophobicity were synthesized in the present work. As shown in Fig. 1, the pyridinium ring and the alkyl groups of the tags render it with strong hydrophobicity, with a calculated Log P value of -3.685 and -2.829 for IMP and IPP, respectively. The embedded nitrogen atom renders the tags with high gas-phase basicity, with a calculated gas-phase hydrogenation capacity of -610.78 kJ/mol and -615.04 kJ/mol for IMP and IPP, respectively. Once peptides are derivatized by IMP or IPP, strong gas-phase basicity and high hydrophobic characteristics could be introduced, benefiting for subsequent MS determination.

3.2. Labeling efficiency and stability

Model peptides CDPGYIGSR, LEACTFRRP, MECFG, and ALVCEQEAR were used to evaluate the labeling efficiency of the developed tags via MALDI-TOF MS. For IMP derivatization, peptide CDPGYIGSR of which the thiol group located in the N-terminal was firstly analyzed. It could be seen that, under the optimal derivatization conditions, the peak representing the native peptide was completely disappeared, and converted into the corresponding derivative, indicating a near 100% derivatization yield (Fig. 2a). Furthermore, for peptides LEACTFRRP, MECFG, and ALVCEQEAR of which the thiol groups located in the interior of the sequences, a near 100% derivatization yield was also achieved under the same labeling conditions (Fig. 2b-d). For IPP derivatization, expect that the derivatization yield of ALVCEQEAR was 99.9%, a complete derivatization was also achieved for peptides

CDPGYIGSR, LEACTFRRP, and MECFG, as shown in Fig. S1. The above results demonstrated that high labeling efficiency was achieved with the developed tags.

To investigate the stability of the peptide derivatives, IMP or IPP derivatized peptide CDPGYIGSR was analyzed. The peptide derivatives were respectively stored in the reaction buffer for 1 h, 6 h, 24 h, 72 h, and 168 h and then analyzed via MALDI-TOF MS. As shown in Fig. S2 and S3, even though the peptide derivatives deposited in the room temperature for 7 days, the MALDI-TOF MS profiling showed no apparent change, indicating high stability of the peptide derivatives.

3.3. Effect of derivatization on MALDI-TOF MS

MALDI-TOF MS instrument is one of the most commonly used mass spectrometer for peptide analysis. Thus, the effect of derivatization of IMP and IPP on the MALDI-TOF MS was firstly investigated and the results were directly compared with that labeled by IAA. Peptides CDPGYIGSR, LEACTFRRP, MECFG, or ALVCEQEAR were respectively derivatized by IMP and IPP and subsequently equimolar mixed with the IAA modified counterparts, followed by MALDI-TOF MS analysis. As shown in Fig. 3 and Fig. S4, for peptides CDPGYIGSR, LEACTFRRP, and ALVCEQEAR, the ionization efficiency was respectively increased about 48.9, 54.3, and 7.2-fold via IMP derivatization. When these peptides were modified by IPP, a more apparent improvement of ionization was achieved and the ionization efficiency of peptides CDPGYIGSR, LEACTFRRP, and ALVCEQEAR was respectively increased 66.1, 66.0, and 24.9-fold compared with that labeled by IAA. The improvement of ionization efficiency could attribute to the increased gas-phase basicity and hydrophobicity of the derivatized peptides which could promote their ionization in MS. Compared with IMP (Gas-phase hydrogenation capacity: -610.78 kJ/mol, Log P: -3.685), IPP possessed higher gas-phase basicity (Gas-phase hydrogenation capacity: -615.04 kJ/mol) and hydrophobicity (Log P: -2.829). Thus, the ionization efficiency increment of the peptides

derivatized by IPP outperformed that derivatized by IMP. Especially, for peptide MECFG, even though the signal to noise (S/N) ratio of the peptide derivatized via IPP reached to 191.5, the peptide peak attributing to the IAA modified species still could not be observed from the mass spectra.

To evaluate the suitability of the developed tags for collision-induced dissociation (CID) fragmentation, model peptide CDPGYIGSR respectively derivatized by IAA, IMP, or IPP was further analyzed via MALDI-TOF MS/MS. As shown in Fig. S5, total 8 fragment ions (4 b ions and 4 y ions) were recognized from the peptide derivatized by IAA, while respectively 9 (4 b ions and 5 y ions) and 10 (5 b ions and 5 y ions) fragment ions were identified from the peptide derivatized by IMP and IPP. Thus, the product ions could be used for the deduction of peptide sequences.

3.4. Effect of derivatization on ESI MS

The effect of derivatization on the ESI MS was further investigated. Firstly, the effect of derivatization on the ESI MS response was studied. As shown in Fig. 4a, compared with IAA modified counterparts, the ionization efficiency of the peptides CDPGYIGSR, LEACTFRRP and ALVCEQEAR derivatized by IMP increased about 1.3, 1.1, and 1.3-fold, respectively. However, when they were labeled by IPP, the ionization efficiency of the peptides was respectively 114.9, 87.0, and 2.2 times higher than that labeled by IAA. Thus, in ESI MS, IPP was also a more efficient tag than IMP for achieving high efficient ionization of peptides, which is consistent with the trend in MALDI-TOF MS. Interestingly, for peptide MECFG, the most obvious increment of ionization efficiency could be observed; the detection sensitivity increased 369.7-fold via IPP derivatization, which further demonstrated that IPP was an more efficient tag than IMP for high sensitive detection of peptides by ESI MS.

Since peptides with high charge states are benefit for high confident identification, especially in the HRMS. Thus, the effect of derivatization on the charges states of these

peptides was further investigated. The average charge states of the peptides were calculated based on a previous report [13]. As shown in Fig. 4b, the average charge states of the peptides CDPGYIGSR, LEACTFRRP, MECFG, and ALVCEQEAR derivatized by IAA were respectively 1.83, 1.88, 1.00, and 1.96. However, the charge states of these peptides increased to 2.13, 2.88, 1.86, 2.70 via IMP derivatization and 2.10, 3.73, 1.78, 2.74 via IPP derivatization. Thus, an increment ranging from 14.8% to 98.4% was achieved, indicating that the charge states of these peptides could also be simultaneously increased via IMP or IPP derivatization.

3.5. Derivatization of proteins

To further indicate the suitability of the developed tags for more complex sample, protein BSA and α-transferrin were further derivatized via IMP or IPP, and the results was also compared with IAA modified counterparts. For BSA, when it was derivatized by IAA, a total number of 26 thiol-containing peptides were identified via MALDI-TOF MS with a single run (Fig. 5a). However, when it was modified by IMP or IPP, as shown in Fig. 5b and 5c, totally 29 and 28 thiol-containing peptides were recognized, among which peptides R. LCVLHEK.T, K,SLHTLFGDELCK.V, K.YICDNQDTISSK.L derivatized by IMP and K.QNCDQFEK.L, K.SLHTLFGDELCK.V, R.RPCFSALTPDETYVPK.A derivatized by IPP were identified as the three most sensitive peaks in the mass spectra. By combination with three consecutive runs, totally 27 thiol-containing peptides were identified via IAA derivatization. When it was modified by IMP or IPP, respectively 32 and 33 thiol-containing peptides were recognized, of which 11 and 13 peptides could not be detected via IAA derivatization (Table 1). Thus, by combination with IMP or IPP derivatization, the identification efficiency of thiol-containing peptides could be respectively increased more than 40% (11/27) or 48% (13/27). These results indicated that high efficient thiol-containing peptides identification could be achieved by combination with IMP or IPP derivatization in

relatively complex sample. Furthermore, to investigate the labeling efficiency of the developed tags for more complex sample, the thiol-containing peptides recognized from BSA were analyzed. As shown in Table S1, except that some of the peptides, such as K.LKECCDKPLLEK.S (yield: 86.1%), R.MPCTEDYLSLILNR.L (yield: 65.7%), K.CCTESLVNR.R (yield: 87.7%) via IMP derivatization and K.QEPERNECFLSHK.D (yield: 66.2%) via IPP derivatization, displayed relatively low labeling efficiency, the yields of almost all of the peptides were higher than 90%, which further demonstrated the high labeling efficiency of the developed tags.

For protein α-transferrin, by combination with three consecutive runs, totally 26 thiol-containing peptides were identified via IAA derivatization. However, when it was modified by IMP or IPP, a total number of 30 and 28 thiol-containing peptides could be confidently recognized, among which 11 peptides could not be detected via IAA modification (Table S2). Therefore, the identification efficiency of thiol-containing peptides was increased more than 42% (11/26) by combination with IMP or IPP derivatization. These results further verified the excellent characteristics of the developed tags for high efficient thiol-containing peptides determination.

4. Conclusions

In conclusion, novel pyridinium-based iodoacetamide functional tags, including IMP and IPP, were designed, synthesized, and further exploited for thiol-containing peptides analysis by MS. Both of the two tags showed high labeling efficiency, superior stability, improved ionization efficiency and increased charge states towards the model peptides, of which IPP, designed with higher gas-phase basicity and hydrophobicity, exhibited the most obvious improvement of ionization efficiency by both MALDI-TOF MS and ESI MS. By further analysis of proteins BSA and α-transferrin, compared with the commonly used alkylation tag IAA, improved identification efficiency of thiol-containing peptides were achieved by

combination with IMP or IPP derivatization. We anticipate the novel tags will be promising for high-efficiency thiol-containing peptides identification, especially for those with low abundance.

Acknowledgments

We are grateful for the financial support from National Natural Science Foundation of China (21205027), Natural Science Foundation of Hebei Province (B2012201052), Young Talent Program in College and University of Hebei Province (B2015201016), and Natural Science Foundation of Hebei University (2011-225).

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Table 1 The recognized thiol peptides from BSA respectively derivatized by IAA, IMP or IPP.

No	Position	Peptide sequence	No of cysteines	IAA	IMP	IPP
1	76-88	K.TCVADESHAGCEK.S	2		√	√
2	89-100	K.SLHTLFGDELCK.V	1	√	√	√
3	106-117	R.ETYGDMADCCEK.Q	2		√	√
4	118-130	K.QEPERNECFLSHK.D	1	√	√	√
5	118-138	K.QEPERNECFLSHKDDSPDLPK.L	1	√	√	√
6	123-130	R.NECFLSHK.D	1		√	√
7	123-138	R.NECFLSHKDDSPDLPK.L	1	√	√	√
8	139-151	K.LKPDPNTLCDEFK.A	1	√	√	√
9	139-155	K.LKPDPNTLCDEFKADEK.K	1	√	√	√
10	139-156	K.LKPDPNTLCDEFKADEKK.F	1	√	√	√
11	184-197	K.YNGVFQECCQAEDK.G	2	√		√
12	184-204	K.YNGVFQECCQAEDKGACLLPK.I	3	√		
13	198-204	K.GACLLPK.I	1		√	√
14	223-228	R.CASIQK.F	1		√	√
15	223-232	R.CASIQKFGER.A	1	√		
16	264-285	K.VHKECCHGDLLECADDRADLAK.Y	3	√		
17	267-285	K.ECCHGDLLECADDRADLAK.Y	3	√	√	√
18	286-297	K.YICDNQDTISSK.L	1	√	√	√

19	298-309	K.LKECCDKPLLEK.S	2	√	√	√
20	300-309	K.ECCDKPLLEK.S	2		√	√
21	310-318	K.SHCIAEVEK.D	1		√	√
22	310-340	K.SHCIAEVEKDAIPENLPPLTADFAEDKDVCK.N	2	√	√	
23	319-340	K.DAIPENLPPLTADFAEDKDVCK.N	1	√	√	√
24	375-386	K.EYEATLEECCAK.D	2	√	√	√
25	387-399	K.DDPHACYSTVFDK.L	1	√	√	√
26	387-401	K.DDPHACYSTVFDKLK.H	1	√	√	√
27	413-420	K.QNCDQFEK.L	1	√	√	√
28	452-468	R.SLGKVGTRCCTKPESER.M	2	√		
29	456-468	K.VGTRCCTKPESER.M	2			√
30	460-468	R.CCTKPESER.M	2			√
31	469-482	R.MPCTEDYLSLILNR.L	1	√	√	√
32	483-489	R.LCVLHEK.T	1		√	√
33	483-495	R.LCVLHEKTPVSEK.V	1		√	√
34	496-507	K.VTKCCTESLVNR.R	2		√	√
35	499-507	K.CCTESLVNR.R	2	√	√	√
36	508-523	R.RPCFSALTPDETYVPK.A	1	√	√	√
37	529-544	K.LFTFHADICTLPDTEK.Q	1	√	√	
38	569-587	K.TVMENFVAFVDKCCAADDK.E	2	√		
39	581-597	K.CCAADDKEACFAVEGPK.L	3	√	√	√
40	588-597	K.EACFAVEGPK.L	1		√	√

Fig. 1 Procedures for synthesis of IMP, IPP and their chemical structures.

Fig. 2 MALDI-TOF MS spectra of the peptides CDPGYIGSR (a), LEACTFRRP (b), MECFG (c), and ALVCEQEAR (d) derivatized by IMP.

Fig. 3 Effect of derivatization of IMP and IPP on the ionization of the peptides via MALDI-TOF MS. The Y axis represents the ratio of S/N of the peptides derivatized by IMP or IPP to the S/N of IAA modified counterparts. *The data represented the S/N values of the peptide derivatized by IMP or IPP.

Fig. 4 Effect of derivatization of IMP and IPP on the ionization (a) and charge states (b) of the peptides via ESI MS.

Fig. 5 MALDI-TOF MS spectra of tryptic digests of BSA respectively derivatized by IAA (a), IMP (b), and IPP (c).

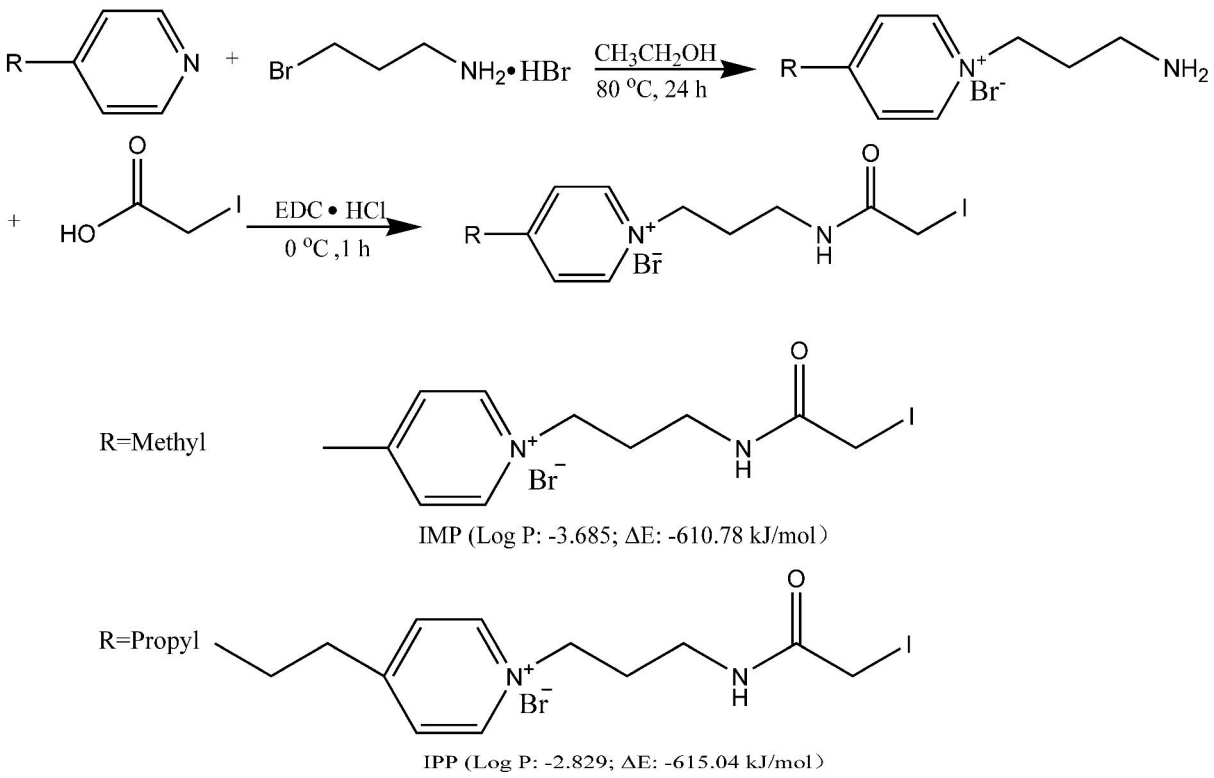

Fig. 1

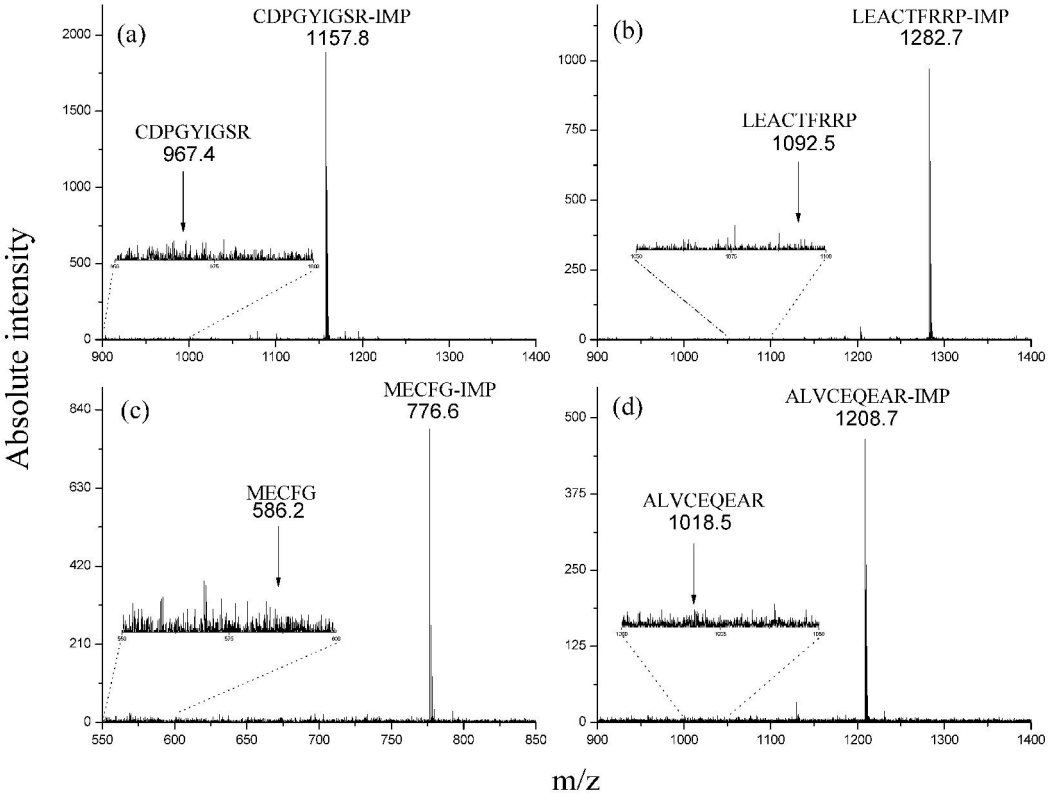

Fig. 2

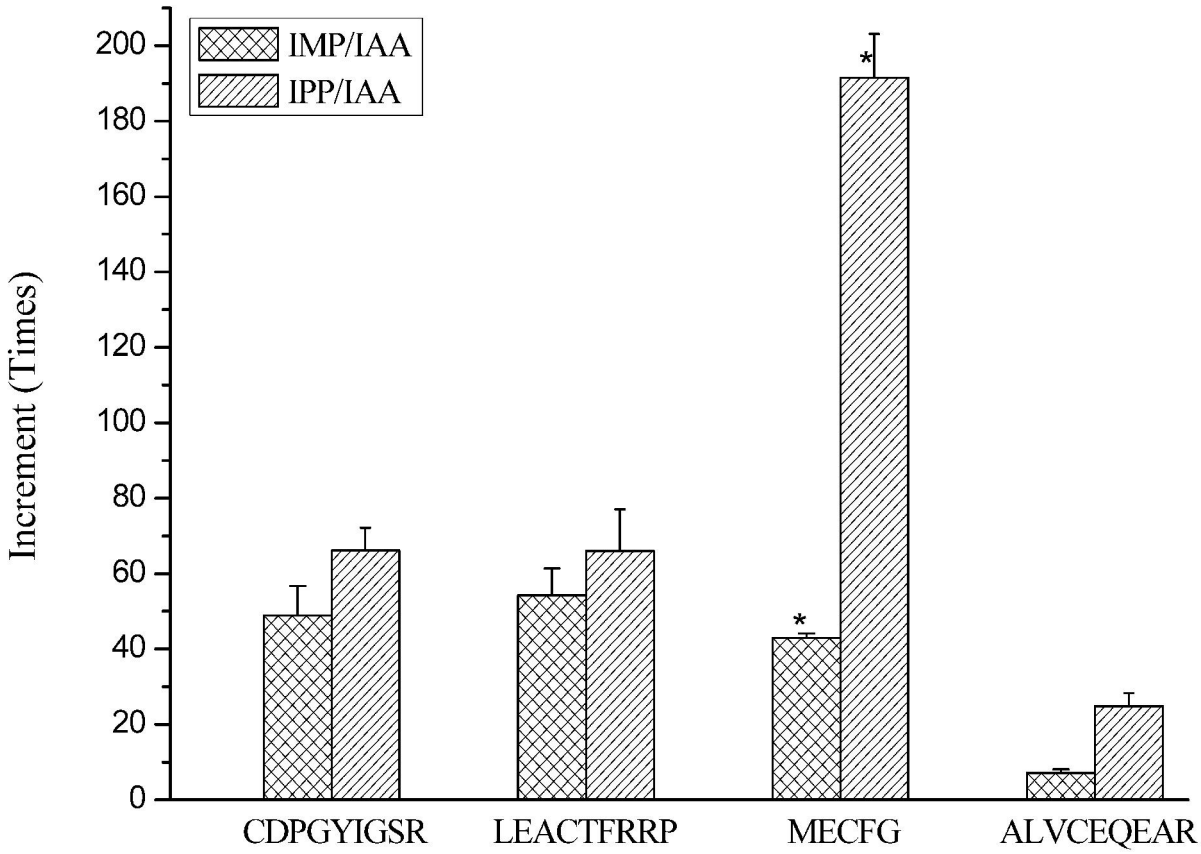

Fig. 3

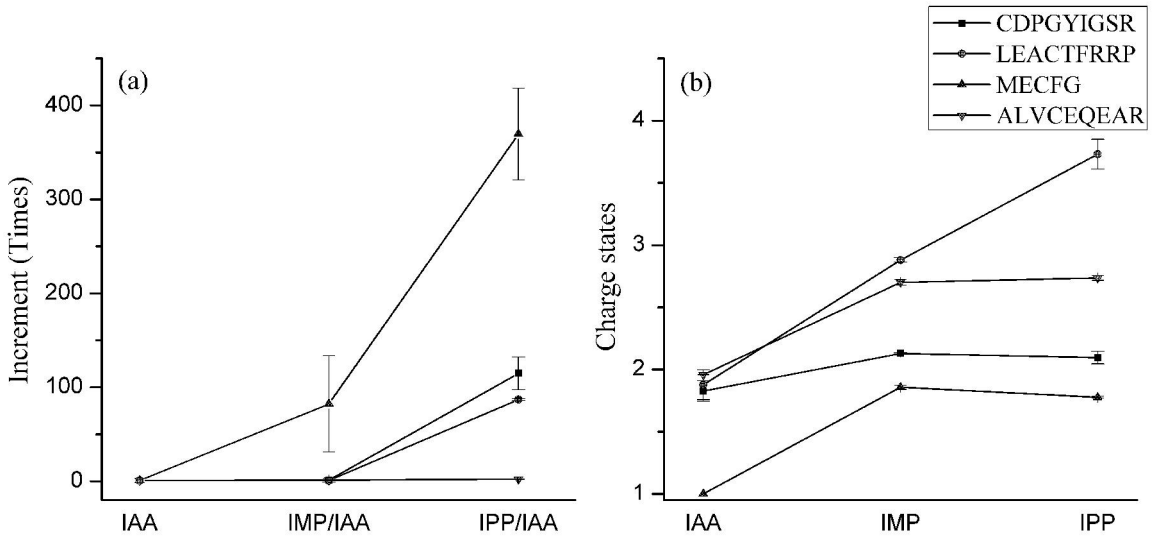

Fig. 4

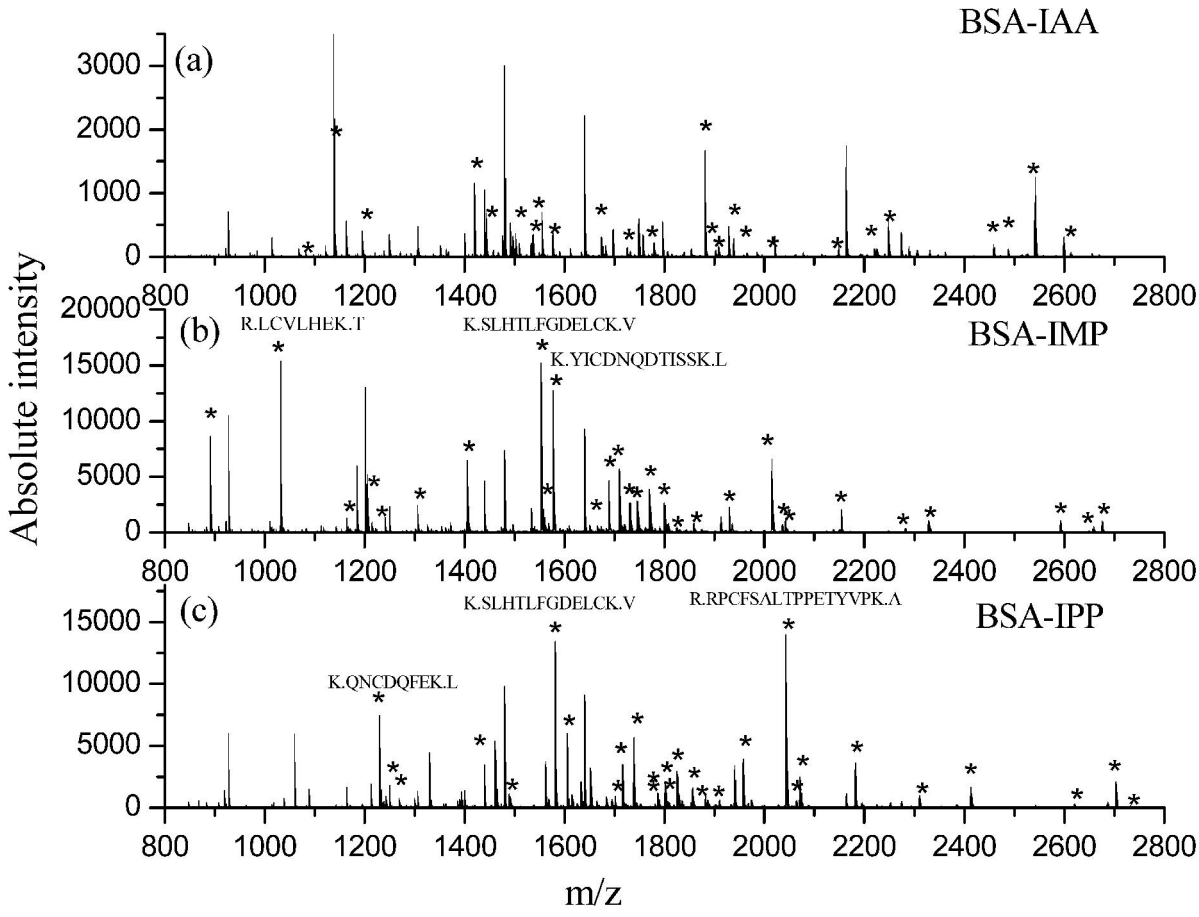

Fig. 5