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# Development of a novel imidazolium-based aromatic quaternary ammonium tag: Synthesis and application to the efficient analysis of cysteinyl-peptides by mass spectrometry

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**RATIONALE:** Chemical derivatization is a very promising technique for improving analysis of peptides by mass spectrometry (MS). In this study, a novel kind of imidazolium-based aromatic quaternary ammonium tag, 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-3-butylimidazolium bromide (IPBI), designed with strong gas-phase basicity and a permanent positive charge, was firstly synthesized and further used for derivatization of cysteinyl-peptides with improved ionization efficiency and higher charge states.
**METHODS:** Both the model peptides and tryptic digests of proteins were used to evaluate the effect of IPBI derivatization on the MS performance of the derivatized peptides, and the results were further compared with the commonly used iodoacetamide (IAA) tag. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)-MS and electrospray ionization (ESI)-MS were used to evaluate the ionization efficiency and charge states of the derivatized peptides.
**RESULTS:** With model peptides as samples, a nearly 100% derivatization efficiency and superior stability were achieved via IPBI derivatization. By further analysis of both standard peptides and tryptic protein digests, the ionization efficiency and charge states of IPBI-derivatized peptides could be remarkably improved. For example, for protein bovine serum albumin, compared with the commercial available IAA tag, the identification efficiency of cysteinyl-peptides was increased about 67% by combining with IPBI derivatization.
**CONCLUSIONS:** The results indicated that the novel tag is an effective derivatization reagent for cysteinyl-peptide identification. We hope it could be further used for high-efficiency cysteinyl-peptide identification in proteome research, especially those with low abundance and poor ionization efficiency. Copyright © 2013 John Wiley & Sons, Ltd.

In the post-genomic era, protein research has been paid much attention. Because of the superior sensitivity, accuracy, and ability to provide structural information, the mass spectrometry (MS)-based technique has increasingly become the analytical method of choice in the field of proteome research in recent years.[1–4] However, for comprehensive peptide/protein profiling, a major limitation of MS-based proteomics is the inefficient and differential ionization of peptides with different size, charge, hydrophobicity, and secondary structure.[5] In particular, the high complexity and tremendous dynamic range of real samples (i.e. human plasma) render the detection of low-abundance peptides still a challenge.[6] Therefore, the development of highly sensitive detection technology for the analysis of peptides with low-abundance or low-ionization efficiency is crucial in proteome research.

Chemical derivatization has long been employed in the field of MS for various purposes, thereby dramatically expanding the usefulness of the measurements.[7–9] More recently, various tagging reagents have been developed and further used for derivatization of the amino group,[10–15] the carboxyl group,[16–21] the hydroxyl group[22,23] on peptides, as well as those with post-translational modifications,[24,25] allowing the analysis of peptides with improved ionization efficiency thereby increased sensitivity. Aldehyde-based tags, butanal and hexanal, were developed by Kulevich *et al.* for the

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derivatization of primary amines to increase the hydrophobicity of peptides. By analyzing both the unmodified and butanal-modified tryptic digests, the combined sequence coverage of bovine serum albumin (BSA) increased by 7%, providing complementary peptide identification.[11] Ko and Brodbelt reported the derivatization of the carboxyl groups of peptides with benzylamine, 1-benzylpiperazine, carboxymethyl trimethylammonium chloride hydrazide, and (2-aminoethyl) trimethylammonium chloride hydrochloride (AETMA). The improved electron transfer dissociation (ETD) efficiencies and greater c- and z-ion populations were obtained via derivatization, especially for the fixed charge reagent, AETAM.[20]

Recently, cysteine has been paid much attention due to its high reactivity, low abundance, and universal distribution in a variety of proteomes.[26] Biological thiols, such as glutathione and thiol proteins, are critical physiological components of fluids and tissues, involved in a variety of important biological events, such as cellular redox signaling and antioxidant defenses, modulation of various cellular activities, and so on.[27] To increase the ionization efficiency of cysteinyl-peptides, a variety of hydrophobic alkyl tags, such as 2-iodo-*N*-octylacetamide, 2-iodo-*N*-dodecylacetamide and 2-iodo-*N*-benzylacetamide, were developed.[28,29] For example, the limit of detection of B-type Natriuretic Peptide was reduced ~3.5-fold via 2-iodo-*N*-octylacetamide derivatization.[30] Zabet-Moghaddam *et al*. reported, using hydrophobic alkyl tag iodoacetanilide (IDA) for derivatization of cysteinyl-peptides, a 4.5-6-fold increment in the peak intensities for model peptides.[31] Quaternary ammonium tags[32] could introduce a permanent positive charge to the modified peptides, benefiting for the analysis of peptides difficult to protonate in MS. For example, a quaternary ammonium tag, (3-acrylamidopropyl)-trimethylammonium chloride (APTA), was developed by Vasicek and coworkers for alkylation of cysteinyl-peptides prior to ETD analysis. The Sequest score of tryptic digests of BSA increased by 6-fold via APTA derivatization, allowing highly credible identification.[33] Shimada *et al*. synthesized several iodoacetic acid-based tags to improve the analysis of cysteinyl-peptides in matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF)-MS, and the synthetic tag, 8-iodoacetoxy-3,6-dioxaoctyltrimethylammonium iodide, incorporating quaternary and hydrophilic linker moieties, showed an optimal sensitivity-enhancing effect for the derivatized peptides.[34] Although a variety of tags have been developed for thiol group derivatization in peptides, the improvement in ionization efficiency is relatively limited and often peptide-dependent. Therefore, the development of novel tags is an imperative task for highly efficient cysteinyl-peptide identification.

Herein, an imidazolium-based aromatic quaternary ammonium tag, 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-3-butylimidazolium bromide (IPBI), designed with strong gas-phase basicity and a permanent positive charge, was synthesized and further exploited for the derivatization of thiol groups in peptides. Through the analysis of both standard peptides and tryptic protein digests, our results demonstrate that highly efficient cysteinyl-peptide identification can be achieved by MALDI-TOF MS and electrospray ionization (ESI)-MS.

## EXPERIMENTAL

### Chemicals and reagents

1-Butylimidazole, iodoacetic acid, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), iodoacetamide (IAA), catalase from bovine liver, BSA, and TPCK-treated trypsin (from bovine pancreas) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-Bromopropylamine hydrobromide was obtained from Aladdin reagent (Shanghai, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and peptides with sequences CDPGYIGSR, ALVCEQEAR, MECFG, and KEEPPHHEVPESETC were from GL Biochem (Shanghai, China). Trifluoroacetic acid (TFA) was purchased from Acros Organics (Geel, Belgium). α-Cyano-4-hydroxycinnamic acid (CHCA) was obtained from Bruker Daltonics (Bremen, Germany). HPLC-grade acetonitrile was from Merck (Darmstadt, Germany). All inorganic reagents were analytical-reagent grade and other reagents were of HPLC grade. Water was purified by a Milli-Q system (Millipore, Molsheim, France).

### Synthesis of 1-(3-aminopropyl)-3-butylimidazolium bromide

The synthetic procedure for 1-(3-aminopropyl)-3-butylimidazolium bromide was according to the previous report.[35] In brief, 1-butylimidazole (1.0 g, 8 mmol) and 3-bromopropylamine hydrobromide (1.2 g, 5 mmol) were firstly dissolved in 10 mL of anhydrous ethanol, followed by refluxing at 80 °C for 24 h under nitrogen atmosphere. Subsequently, ethanol was removed under vacuum and the crude residue was dissolved in 2 mL of water that was brought to pH ~8 by the addition of solid potassium hydroxide. Finally, the water was removed under vacuum again, and the crude residue was redissolved in 30 mL of tetrahydrofuran/methanol (1:1, v/v), filtered, dried under vacuum, and thoroughly washed with dichloromethane to yield the final product (yield, 75%).

### Synthesis of IPBI

1-(3-Aminopropyl)-3-butylimidazolium bromide (125 mg, 0.48 mmol) was firstly dissolved in 2 mL of acetonitrile/water (1:4, v/v) and the resulting mixture was stirred at 0 °C for 10 min. Then, iodoacetic acid (54 mg, 0.21 mmol) and EDC·HCl (68 mg, 0.35 mmol) were sequentially added, and the resulting mixture was stirred at 0 °C for an additional 1 h. Finally, the crude product was purified by reversed-phase HPLC with acetonitrile/water containing 0.1% (v/v) TFA as the mobile phase to yield the final product (yield, 73%). Fourier transform infrared (FT-IR) measurement was performed using a Bruker Vertex 70 spectrometer (Bruker Optik GmbH, Ettlingen, Germany) in the transmission mode. NMR spectrum was obtained on a Bruker AVANCE III 600 MHz spectrometer (Bruker, Bremen, Germany), and high-resolution (HR) MS spectrum was determined on a Bruker apex ultra 7.0 T Fourier transform mass spectrometer (Bruker, Bremen, Germany). $^1$H NMR (600 MHz, DMSO) $\delta = 9.15$ (s, 1H), 7.17 (s, 1H), 7.09 (s, 1H), 7.00 (s, 1H), 4.19–4.16 (m, 4H), 3.63 (s, 2H), 1.98–1.94 (m, 2H), 1.79–1.76 (m, 2H), 1.27–1.23 (m, 4H), 0.91 (t, 3H, $J = 7.4$ Hz). HRMS, *m/z*:



  

350.0719. The HRMS, NMR, and FT-IR spectra are shown in Supplementary Figs. S1, S2, and S3 (see Supporting Information).

### Peptide derivatization

In a typical experiment, 5 μL of 1 mg/mL peptides ALVCEQEAR, CDPGYIGSR, KEEPPHHEVPESETC or 0.5 mg/mL MECFG was initially mixed with 20 μL of 10 mM TCEP in 50 mM Tris-HCl buffer (pH ~8.4). After the peptides were reduced at 50 °C for 1 h, an aliquot of 50 μL of 50 mM IPBI was added, followed by incubation at 37 °C in the dark for 2 h with end-over-end rotation. The derivatized peptides could be directly applied for MALDI-TOF MS or ESI-MS analysis without any additional cleanup step. The alkylation procedure of the peptides with IAA or IDA was similar to the peptide derivatization experiment, except that 50 mM of IPBI was replaced by 50 mM of IAA or IDA.

### Protein derivatization and digestion

In brief, 1.0 mg of protein catalase or BSA was firstly dissolved in 70 μL of 8 M urea and then denatured at 56 °C for 1 h. Subsequently, 70 μg of proteins were reduced by 10 mM TCEP at 50 °C for 1 h. After cooling to room temperature, 50 μL of 50 mM IPBI were added, followed by further incubation at 37 °C in the dark for 2 h with end-over-end rotation. After the proteins were desalted and further re-dissolved in 300 μL of 60 mM Tris-HCl buffer (pH 8.3), trypsin was added with enzyme/protein ratio of 1:20 (w/w). The sample was incubated at 37 °C overnight, ready for analysis. The alkylation procedure of the protein with IAA was similar to the derivatization procedure, except that 50 mM of IPBI was replaced by 50 mM of IAA.

### HPLC analysis

HPLC experiments were performed using a P230II system and a Sinchrom ODS-AP column (5 μm, 300 Å, 4.6 × 250 mm i.d., Dalian Elite Analytical Instruments, Dalian, China). Eluent A was 100% water with 0.1% (v/v) TFA; eluent B was 100% acetonitrile with 0.1% (v/v) TFA. The gradient was set as follows: 0 min, 5% B, 13 min, 31% B, with a flow rate of 1.0 mL/min. The UV wavelength was 214 nm.

### MS analysis

MALDI-TOF MS experiments were performed on a Bruker Ultraflex III TOF/TOF mass spectrometer (Bruker, Bremen, Germany) in positive ion reflectron mode. An aliquot of 1 μL of native or derivatized peptides was directly deposited and dried on the polished steel target, followed by 1 μL of matrix solution (7 mg/mL CHCA in 0.1% TFA/60% acetonitrile) deposition. The instrument was immediately calibrated using standard peptides before experiments. MS spectra were processed and analyzed with the software FlexAnalysis (version 3.0) and BioTools (version 3.2) (Bruker, Bremen, Germany). The protein was identified by peptide mass fingerprinting (PMF), and database searching was performed on the MASCOT server (Matrix Science, London, UK) to search the database of bovine, with parameters as following: (i) enzyme: trypsin; (ii) fixed modification: carbamidomethyl (C) or modifying cysteine residue with 222.16064 Da for IPBI derivatization; (iii) variable modification: oxidation (M); (iv) peptide mass tolerance: 200 ppm; (v) missed cleavages: 2.

A Finnigan surveyor MS pump coupled with a LTQ linear ion trap mass spectrometer (both Thermo Finnigan, San Jose, CA, USA) were used for ESI-MS determination.

## RESULTS AND DISCUSSION

Gas-phase basicity and hydrophobicity are crucial factors affecting the ionization of analytes by MS.[5,36] In the present study, a novel kind of imidazolium-based aromatic tag was designed and synthesized by a quaternization reaction (Fig. 1(a)). The nitrogen atoms in imidazole give the tag strong gas-phase basicity, while the introduced *n*-butyl and *n*-propyl groups could attenuate the hydrophilicity of the quaternized cation tag. These structural characteristics are particularly beneficial for subsequent MS analysis. By further activation with iodoacetic acid, the tagging agent could readily react with the thiol group of cysteine via alkylation derivatization (Fig. 1(b)).

### Derivatization efficiency

The model peptide CDPGYIGSR with the cysteine residue located in the N-terminal part was firstly used to evaluate the derivatization efficiency. Figure 2(a) shows the representative chromatograms of native peptide CDPGYIGSR and that

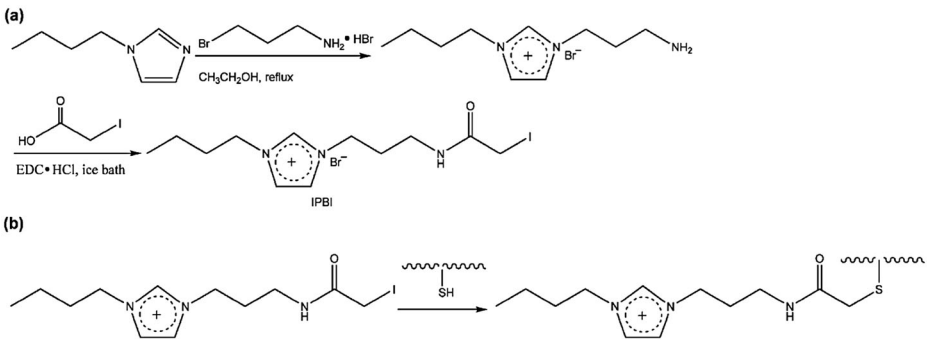


**Figure 1.** Scheme of IPBI synthesis (a) and peptide/protein derivatization (b).

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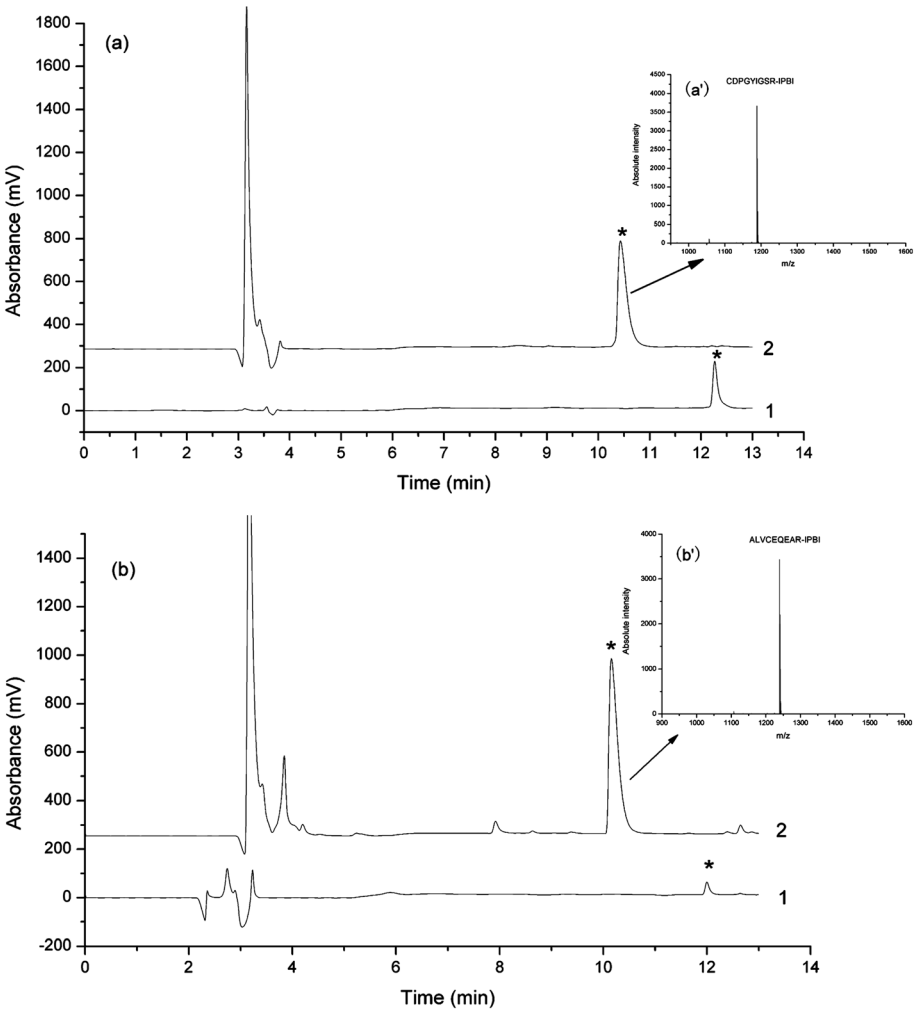


**Figure 2.** Chromatograms of peptides CDPGYIGSR (a) and ALVCEQEAR (b) before (1) and after (2) IPBI derivatization. a′ and b′ are the MALDI-TOF MS spectra of IPBI-derivatized peptides. Peaks* are the peptide peaks.

derivatized by IPBI. It can be seen that, under the optimal derivatization conditions, the peak representing the native peptide had fully disappeared and converted into the corresponding derivatized product. The peptide derivative was further verified via MALDI-TOF MS (Fig. 2(a′)). The cysteine residues located in the interior domain of the peptide possessing relative large steric hindrance are more difficult to thoroughly label during the derivatization procedure. Therefore, the model peptide with the sequence ALVCEQEAR was further used to evaluate the derivatization efficiency. Similarly, the native peptide peak completely disappeared and converted into the corresponding derivative (Fig. 2(b)), indicating a 100% derivatization efficiency. The above results demonstrated that high derivatization efficiency could be achieved by the developed tag.

The stability of peptide derivatives was further investigated with the model peptide ALVCEQEAR as the sample. As shown in Supplementary Fig. S4 (Supporting Information), even though the IPBI-derivatized peptides were stored at room temperature for up to 1 week, no noticeable change in the MALDI-TOF MS profiling was observed, indicating its good stability. As extra reagents would not induce a side-chain reaction, therefore, no additional cleanup step is needed before MS analysis.

### Effect of derivatization on ionization efficiency

IAA is the most commonly used tag for thiol alkylation in proteome research. Thus, the effect of derivatization on the ionization efficiency of peptides via IPBI was firstly compared with the IAA-modified counterpart. For the model peptides CDPGYIGSR and ALVCEQEAR, an aliquot of IPBI-derivatized peptide was respectively mixed with its IAA-modified counterpart in equimolar quantities and subsequently subjected to MALDI-TOF MS analysis. According to the representative MALDI-TOF MS spectra shown in Figs. 3(a) and 3(b), the signal-to-noise (S/N) ratios of IPBI-derivatized peptides were respectively 62 and 97 times (n = 5) higher than that of those labeled with commercial IAA. For peptides MECFG and KEEPPHHEVPESETC, most strikingly, even though the S/N ratios of the peptides derivatized via IPBI reached 278 and 1250, respectively, the peptide peaks representing the IAA-modified species were still not observable (Figs. 3(c) and 3(d)), indicating that the ionization efficiency of the peptides derivatized via IPBI was largely increased.

To further evaluate the effect of derivatization on ionization efficiency, peptides derivatized by IPBI were also compared with its native cognates. For peptides CDPGYIGSR and



  

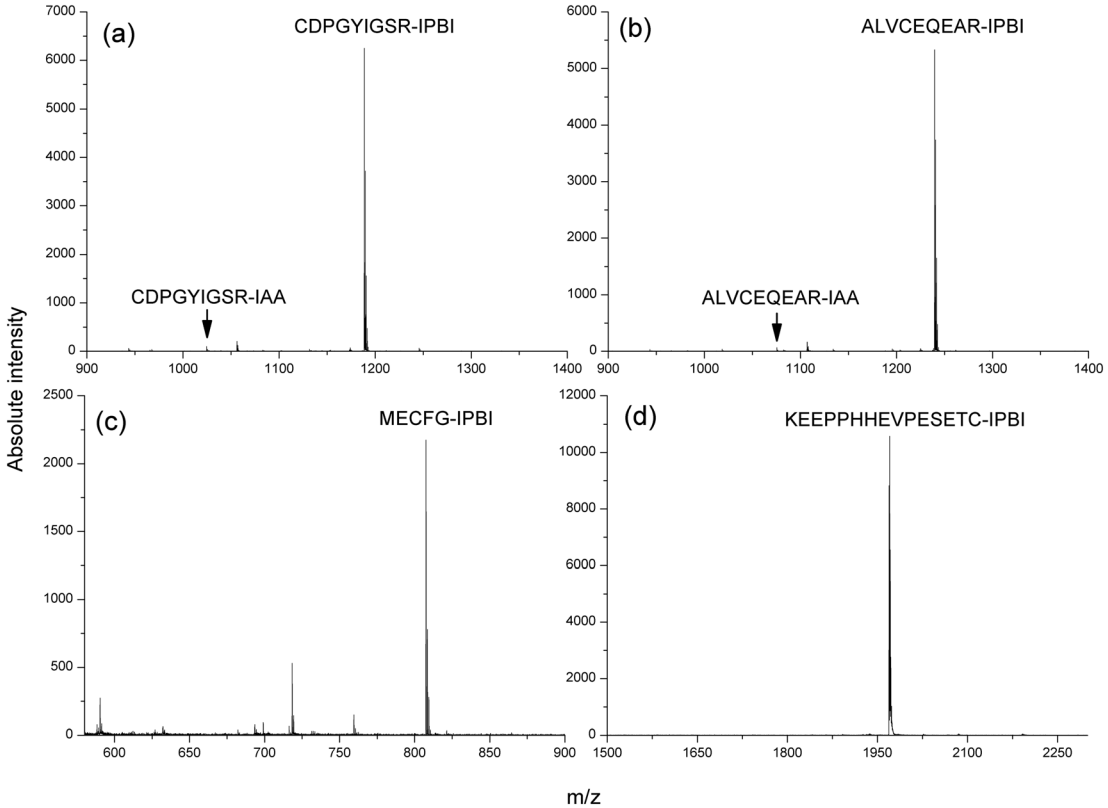


**Figure 3.** MALDI-TOF mass spectra of equimolar mixtures of IPBI- and IAA-derivatized peptides CDPGYIGSR (a), ALVCEQEAR (b), MECFG (c), and KEEPPHHEVPESETC (d).

ALVCEQEAR, the ionization efficiency was respectively increased by 100- and 114-fold (n = 5) via IPBI derivatization (Table 1 and Supplementary Fig. S5, Supporting Information). Moreover, the developed tag is more efficient than a recently reported tag, IDA,[31] which has been used to increase peptide peak intensities in MALDI-TOF MS. Compared with those labeled by IDA, the ionization efficiency of peptides CDPGYIGSR, ALVCEQEAR, and KEEPPHHEVPESETC were respectively increased by 43-, 4-, and 60-fold (n = 5) via IPBI derivatization (Table 1 and Supplementary Fig. S6, Supporting Information).

Furthermore, in ESI-MS, peptides derivatized by IPBI also showed improved ionization efficiency. For example, even though the ionization efficiency of the peptide ALVCEQEAR is roughly equal to the IAA-modified counterpart, the ionization efficiency of peptides CDPGYIGSR, MECFG, and KEEPPHHEVPESETC were respectively increased by 20-, 627-, and 4-fold via IPBI derivatization, which further demonstrated that IPBI is an efficient tag for highly sensitive peptide detection in MS.

**Table 1.** S/N ratios of model peptides derivatized by IPBI vs that modified by IAA, IDA or the native cognates

| | IPBI/IAA | IPBI/Native | IPBI/IDA |
|---|---|---|---|
| CDPGYIGSR | 62 | 100 | 43 |
| ALVCEQEAR | 97 | 114 | 4 |
| MECFG | 278* | 472* | 180* |
| KEEPPHHEVPESETC | 1250* | 3602* | 60 |

*The data represent the S/N values of the peptides derivatized by IPBI.

### MS/MS analysis of model peptide

To further evaluate the effect of derivatization on collision-induced dissociation (CID) fragmentation, the model peptide CDPGYIGSR was respectively derivatized by IAA and IPBI and further analyzed via MALDI-TOF MS/MS. The MS/MS spectra and the peak map are shown in Fig. 4. It can be seen that four b-type ions and three y-type ions are found from the peptide derivatized via IAA (Fig. 4(a)) while respectively three b-type ions and three y-type ions can be successfully recognized from the peptide derivatized via IPBI (Fig. 4(b)). Thus, the product ions could be used for the deduction of the peptide sequence.

### Effect of derivatization on charge states

Higher charge states are beneficial for the highly confident identification of peptides and proteins by MS, because the ion's S/N ratio, mass resolving power, and mass accuracy are all proportional to the charge states of analytes in the highest resolution mass analyzer (such as ion cyclotron resonance and orbitrap). Furthermore, for the relative novel

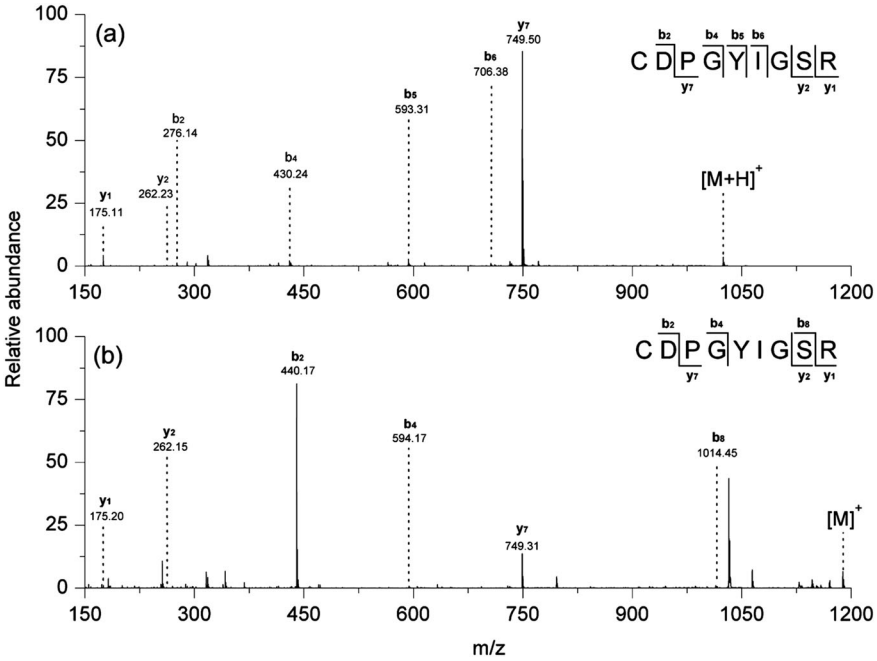


**Figure 4.** CID product ion mass spectra of the peptide CDPGYIGSR derivatized with IAA (a) and IPBI (b).

dissociation model, ETD and electron capture dissociation (ECD), with the increment of the square of ion charge, the MS/MS efficiency could be increased.[37] Therefore, the effect of derivatization on the charge states of peptides was also investigated. The average charge state was calculated using the formula:

$$c_{average} = \sum_{i}^{n} c_i A_i / \sum_{i}^{n} A_i$$

where $n$ represents the number of ion-charge states while $c_i$ and $A_i$ respectively represent the net charge of the $i$-th charge state and the signal intensity of the $i$-th charge state.

Figure 5 shows the representative ESI mass spectra of the peptide CDPGYIGSR derivatized by IAA and IPBI, respectively. When the peptide was modified with IAA, both the doubly charged species and the singly charged species were observed, giving an average charge state of 1.89 (Fig. 5(a)). However, after the peptide had

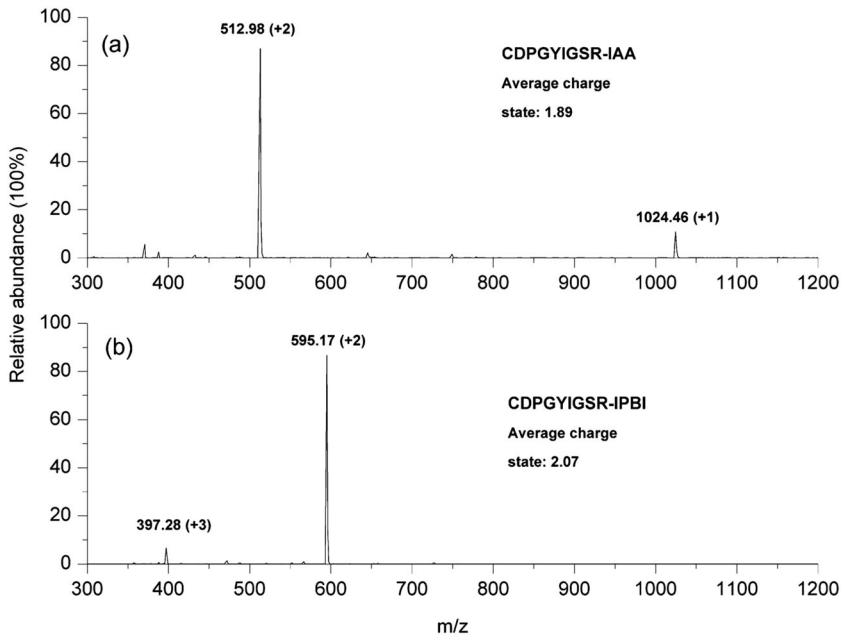


**Figure 5.** ESI mass spectra of peptide CDPGYIGSR respectively derivatized by IAA (a) and IPBI (b).



  

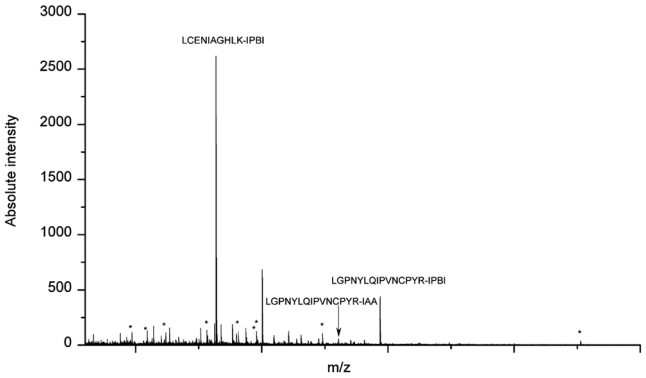


**Figure 6.** MALDI-TOF mass spectrum of equimolar mixtures of tryptic digests of protein catalase respectively derivatized by IAA and IPBI. Peaks* represent the non-cysteinyl-peptides recognized from the protein.

beenderivatized by IPBI, the singly charged species completely disappeared, and the triply charged species could be observed, achieving an average charge state of 2.07 (Fig. 5(b)). A dramatic shift toward higher charge states has also been observed for the peptides ALVCEQEAR, MECFG and KEEPPHHEVPESETC. As shown in Supplementary Fig. S7 (Supporting Information), the average charge state of the peptides derivatized by IPBI could be respectively increased to 2.67, 1.88, and 3.51 from 1.91, 1.00, and 2.94. Thus, the charge states of the derivatized peptides could be simultaneously increased with the developed tag.

### Analysis of tryptic digests of proteins

To evaluate the efficiency of IPBI derivatization on complex samples, a protein catalase containing four cysteine residues was firstly analyzed. The protein was respectively derivatized via IAA and IPBI, digested with trypsin and further analyzed via MALDI-TOF MS. Figure 6 shows the representative MS spectrum of an equimolar mixture of IPBI- and IAA-modified peptides. For IAA derivatization, only one cysteinyl-peptide with the sequence R.LGPNYLQIPVNCPYR.A was recognized. However, after it was modified with IPBI, the two strongest peaks were recognized as the cysteinyl-peptides R.LGPNYLQIPVNCPYR.A and R.LCENIAGHLK.D. Most strikingly, peptide R.LCENIAGHLK.D, which was not even detected via IAA modification, gave the highest signal intensity via IPBI derivatization.

BSA is a more complex protein with 607 amino acid units, of which 35 amino acids are cysteine residues. Thus, the developed tag was further used for the analysis of tryptic digests of BSA, and the results were further compared with the commonly used IAA tag. By combining the three consecutive results, in total 15 cysteinyl-peptides were identified via IAA derivatization (Table 2). However, when it was labeled by IPBI, a total of 21 cysteinyl-peptides were confidently recognized, among which 10 were not even detected via IAA derivatization. Thus, the identification efficiency of cysteinyl-peptides was increased about 67% (10/15) by combining with IPBI derivatization. It clearly shows that highly efficient peptide detection could be achieved by combining with IPBI derivatization in a relatively complex sample.

**Table 2.** The identified cysteinyl-peptides from BSA modified with IAA and IPBI

| No | Position | Peptide sequence | No of cysteines | IAA | IPBI |
|---|---|---|---|---|---|
| 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 | 139-151 | K.LKPDPNTLCDEFK.A | 1 | | √ |
| 7 | 139-155 | K.LKPDPNTLCDEFKADEK.K | 1 | √ | √ |
| 8 | 223-228 | R.CASIQK.F | 1 | | √ |
| 9 | 264-285 | K.VHKECCHGDLLECADDRADLAK.Y | 3 | √ | |
| 10 | 286-297 | K.YICDNQDTISSK.L | 1 | √ | √ |
| 11 | 298-309 | K.LKECCDKPLLEK.S | 2 | √ | √ |
| 12 | 300-309 | K.ECCDKPLLEK.S | 2 | | √ |
| 13 | 310-318 | K.SHCIAEVEK.D | 1 | | √ |
| 14 | 310-340 | K.SHCIAEVEKDAIPENLPPLTADFAEDKDVCK.N | 2 | √ | |
| 15 | 375-386 | K.EYEATLEECCAK.D | 2 | | √ |
| 16 | 387-401 | K.DDPHACYSTVFDKLK.H | 1 | | √ |
| 17 | 413-420 | K.QNCDQFEK.L | 1 | √ | √ |
| 18 | 460-468 | R.CCTKPESER.M | 2 | √ | √ |
| 19 | 483-489 | R.LCVLHEK.T | 1 | | √ |
| 20 | 483-495 | R.LCVLHEKTPVSEK.V | 1 | √ | √ |
| 21 | 483-498 | R.LCVLHEKTPVSEKVTK.C | 1 | √ | |
| 22 | 499-507 | K.CCTESLVNR.R | 2 | √ | √ |
| 23 | 508-523 | R.RPCFSALTPDETYVPK.A | 1 | √ | |
| 24 | 581-597 | K.CCAADDKEACFAVEGPK.L | 3 | √ | √ |
| 25 | 588-597 | K.EACFAVEGPK.L | 1 | | √ |

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## CONCLUSIONS

An imidazolium-based aromatic quaternary ammonium tag, IPBI, was designed, synthesized, and further exploited for cysteinyl-peptide derivatization. The high derivatization efficiency and superior stability of IPBI derivatives could ensure good reproducibility of sample labeling. Once peptides have been labeled with IPBI, the group with strong gas-phase basicity and a permanent positive charge could be introduced. Thus, both the ionization efficiency and charge states of derivatized peptides could be remarkably improved, which was proved by applying the developed tag to derivatize both standard peptides and tryptic protein digests. We expect the novel tag will be very promising in high-efficiency cysteinyl-peptide identification, especially those with low abundance and poor ionization efficiency.

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