SCIENCE CHINA
Life Sciences

SPECIAL TOPIC: Lifeomics and translational medicine
• RESEARCH PAPER •

March 2013 Vol.56 No.3: 240–245
doi: 10.1007/s11427-013-4446-8

1-(3-Aminopropyl)-3-butylimidazolium bromide for carboxyl group derivatization: potential applications in high sensitivity peptide identification by mass spectrometry

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Received July 16, 2012; accepted October 30, 2012

The cationic reagent 1-(3-aminopropyl)-3-butylimidazolium bromide (BAPI) was exploited for the derivatization of carboxyl groups on peptides. Nearly 100% derivatization efficiency was achieved with the synthetic peptide RVYVHPI (RI-7). Furthermore, the peptide derivative was stable in a 0.1% TFA/water solution or a 0.1% (v/v) TFA/acetonitrile/water solution for at least one week. The effect of BAPI derivatization on the ionization of the peptide RI-7 was further investigated, and the detection sensitivity was improved >42-fold via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), thus outperforming the commercial piperazine derivatization approach. Moreover, the charge states of the peptide were largely increased via BAPI derivatization by electrospray ionization (ESI) MS. The results indicate the potential merits of BAPI derivatization for high sensitivity peptide analysis by MS.

1-(3-aminopropyl)-3-butylimidazolium bromide, ionization capacity, peptide, mass spectrometry analysis, derivatization

Citation: Qiao X Q, Zhou Y, Hou C Y, et al. 1-(3-Aminopropyl)-3-butylimidazolium bromide for carboxyl group derivatization: potential applications in high sensitivity peptide identification by mass spectrometry. Sci China Life Sci, 2013, 56: 240−245, doi: 10.1007/s11427-013-4446-8

Significant attention towards protein research has been made in the post-genomic era. However, because of the high complexity and extremely wide dynamic range of proteins, the detection sensitivity of proteins often can barely meet the requirements of real sample analysis. In particular, many proteins with important biological functions are present in low abundance (e.g., biomarkers and drug targets), which prohibits the efficient detection of these proteins [1,2]. Therefore, improving the detection sensitivity of protein/peptides is an important task. Preconcentration is a widely used technique to improve the detection sensitivity of analytes [3,4]. Foote et al. applied a porous silica membrane to concentrate fluorescently labeled proteins and a signal enhancement of ~600-fold was achieved by capillary gel electrophoresis [5]. As a promising alternative, the chemical derivatization strategy has gained in popularity. This approach is widely used for highly efficient protein/peptide analysis and is compatible with a variety of detection techniques [6–10].

Currently, mass spectrometry (MS) based detection techniques have emerged as indispensable tools for peptide analysis [11]. To increase the ionization efficiency of pep-

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tides (especially for those with low abundance and poor ionization efficiency) by MS, a variety of chemical derivatization reagents [12] that target labeling of the amino group [13–15], thiol group [16–18] and hydroxyl group [19,20] have been developed. Smith et al. applied the aldehyde-based derivatization reagent hexanal and butanal for the alkylation of primary amines in peptides to provide an increase in ionization efficiency and concomitant signal enhancement. By combining with the analysis of the modified and unmodified digests, the sequence coverage of cytochrome c, β-lactoglobulin A and bovine serum albumin were significantly increased [14]. Cationic derivatization reagents [9,17,18] always include a permanent positive charge. Therefore, the derivatized peptide should introduce a positive charge that benefits the analysis of peptides that do not adsorb protons readily. Ma et al. applied the cationic tag, 1-[3-(4-maleimidylphenoxy)propyl]trimethylammonium bromide for the derivatization of thiol groups in peptides. The signal intensity of the peptides with more polar residues could be increased about 3–5-fold, whereas those peptides with relative lower abundance of polar residues could be improved more than 100-fold [17]. Brodbelt et al. used the cationic derivatization reagent (3-acrylamidopropyl)-trimethyl ammonium chloride for the alkylation of cysteine-containing peptides. Both of the charge states and the sequence coverage on electron-transfer dissociation (ETD) MS were clearly increased. Through the analysis of tryptic digests of bovine serum albumin, the SEQUEST score increased to 3700 from 582 via derivatization, allowing high credible identification [18].

The carboxyl group based derivatization method possesses potential merits in high-efficiency peptide analysis [21]. Recently, several neutral derivatization reagents [22–24] were exploited and further used for the derivatization of carboxyl groups on peptides. Lu et al. applied neutral 1-(2-pyrimidyl)piperazine for the derivatization of carboxyl groups of phosphopeptides; both the ionization efficiency and enrichment specificity of phosphopeptides were clearly improved [22]. 2-Nitrophenylhydrazine was developed by Ball et al. to derivatize the carboxl groups of peptides and the detection sensitivity of model peptides was improved by 15-fold [23]. In our previous research, piperazine-based derivatives, 1-(2-pyridyl)piperazine, 1-(2-pyrimidyl)piperazine, 1-(4-pyridyl)piperazine and 1-(1-methyl-4-piperidinyl)piperazine were used for peptide derivatization. Peptides with low molecular weight and high pI were preferably detected via 1-(2-pyridyl)piperazine and 1-(2-pyrimidyl) piperazine derivatization [24].

The cationic derivatization reagent 1-(3-aminopropyl)-3-butylimidazolium bromide (BAPI) was explored as a derivatization agent of carboxyl groups on peptides. The results demonstrated that both the ionization efficiency and charge states of the peptide were significantly increased by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS and electrospray ionization (ESI) MS.

1 Materials and methods

1.1 Reagents and materials

1-Butylimidazole, 3-bromopropylamine hydrobromide and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxy-7-azabenzotriazole (HOAt) and the peptide with the sequence RVYVHPI (RI-7) were obtained from GL Biochem (Shanghai, China). Trifluoroacetic acid (TFA) was ordered from Acros organics (Geel, Belgium). α-Cyano-4-hydroxycinnamic acid (CHCA) was from Bruker Daltonics (Bremen, Germany). HPLC-grade acetonitrile was purchased 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).

1.2 Synthesis of BAPI

The synthesis procedure of BAPI was reported previously [25]. 1-Butylimidazole (2.0 g, 16 mmol) and 3-bromopropylamine hydrobromide (3.5 g, 16 mmol) were dissolved in 20 mL of anhydrous ethanol, followed by refluxing at 90°C for 24 h under nitrogen atmosphere. Subsequently, ethanol was removed under vacuum and the residues were redissolved in 3 mL of water. Solid potassium hydroxide was added to adjust the solution pH to ~8. Finally, the product was redissolved in 30 mL of methanol/tetrahydrofuran (1∶1, v/v), filtered, dried under vacuum and purified by reversed-phase high-performance liquid chromatography with acetonitrile-water as the mobile phase.

1.3 Peptide derivatization

In a typical experiment, 7 μL of 0.8% BAPI in DMF was initially mixed with 20 μL of 10 ng μL^{-1} RI-7 in water. Then, 4 μL of 2 mg mL^{-1} EDC in DMF and 3 μL of 2 mg mL^{-1} HOAt in DMF were sequentially added, followed by rigorous mixing at room temperature for 10 s. Finally, 24 μL of 0.03% TFA in water was added and further mixed at room temperature for 10 s. The reaction was terminated by vacuum centrifugation (Thermo Fisher, San Jose, CA, USA), followed by redissolving the derivatized peptide in water or 50% acetonitrile/water containing 0.1% (v/v) TFA. The sample was ready for use. The blank derivatization experiment was similar to the peptide derivatization experiment, except the peptide solution was replaced by water.

1.4 MS analysis

MALDI-TOF MS experiments were performed on a Bruker Ultraflex III TOF/TOF mass spectrometer (Bruker, Bremen, Germany) in the positive ion reflectron mode. An aliquot of

0.5 μL of native or BAPI derivatized peptide solution was deposited and dried on the polished steel target, followed by 1 μL of matrix solution (7 mg mL^{-1} CHCA in 0.1% TFA/60% acetonitrile) deposition. The instrument was immediately calibrated using standard peptides before experiments. ESI MS experiments were performed on a LCQ^{DUO} quadrupole ion trap mass spectrometer (LCQ-IT MS, Thermo Fisher, San Jose, CA, USA).

2 Results and discussion

It is well-known that gas-phase basicity and hydrophobicity are two important factors affecting the ionization of analytes by MS [26,27]. As shown in Figure 1A, the propyl and butyl groups of BAPI render this compound with excellent hydrophobicity, whereas the imidazole amine renders BAPI with higher gas-phase basicity. Furthermore, the quaternary ammonium group of BAPI could introduce a permanent positive charge to peptides, this is particularly beneficial to the analysis of peptides with low charge states. All these characteristics render BAPI as a potential derivatization reagent for high efficient peptide identification by MS.

As shown in Figure 1B, with EDC and HOAt as the coupling reagents, the amino group of BAPI could react with the carboxyl groups of peptides to form a covalent complex. Model peptide RI-7 was used for the evaluation of the derivatization efficiency of BAPI. As shown in Figure 2A, under optimal reaction conditions, the peak representing the native peptide at *m*/*z* 883.5 had completely disappeared in the MALDI-TOF mass spectra, and fully converted into the corresponding product (*m*/*z* 1046.7), suggesting 100% derivatization efficiency. BAPI derivatization resulted in the addition of 164.2 Da molecular mass, which is generated from the conjugation of BAPI (*m*/*z* 182.17) and concomitant loss of one water molecular. The stability of the peptide derivative was further investigated. The derivatization reaction was first terminated by vacuum centrifugation, and the dry sample re-dissolved in water containing 0.1% TFA or 50% acetonitrile/water containing 0.1% TFA. As shown in Figure 2B and C, no noticeable change of the MALDI-TOF MS profiling of BAPI derivatized RI-7 was observed even when it was stored at room temperature for one week, indicating that the BAPI-derivatized peptide shows good stability.

To evaluate the effect of BAPI derivatization on the ionization of peptides, the termination efficiency by vacuum centrifugation was investigated. As shown in Figure 3A, the BAPI derivatized product (*m*/*z* 1046.7) could not be found from the MALDI MS mass spectra by the analysis of the native peptide and the blank derivatization mixture, indicating that extra reagents did not react with the peptide. By further analysis of the equimolar mixed native and BAPI derivatized RI-7, as shown in Figure 3B, the average detection sensitivity of the derivatized peptide was 42.2 times (*n*=5) higher than that of the native peptide. The above result outperforms the result in the latest publication that uses the commercial piperazines derivatization approach [24].

The charge states of the native and BAPI derivatized RI-7 was further analyzed. Figure 4A is a representative ESI mass spectrum of native RI-7; both the single-charged and double-charged species of the peptide were observed. However, after it was modified with BAPI, as shown in Figure 4B, the peak representing the single-charged species had completely disappeared, and the triple-charged species was dominant in the mass spectrum, indicating that the charge states of the peptide were largely increased via BAPI derivatization. Thus, BAPI derivatization is particularly beneficial for the analysis of peptides that do not readily adsorb a proton or in the charge state dependent dissociation model (such as ETD MS).

3 Conclusion

In this work, the cationic derivatization reagent BAPI was exploited for peptide carboxyl derivatization. The high labeling efficiency and excellent stability ensured good reproducibility of the sample derivatization. In addition, not only the detection sensitivity of the peptide was largely enhanced, but also the charge states were clearly increased.

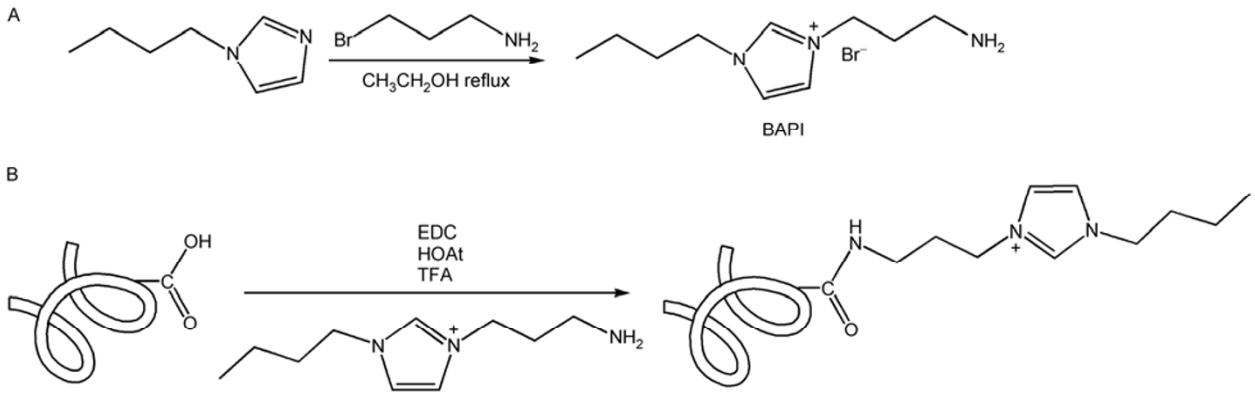

Figure 1 Scheme of BAPI synthesis [25] (A) and peptide derivatization (B).

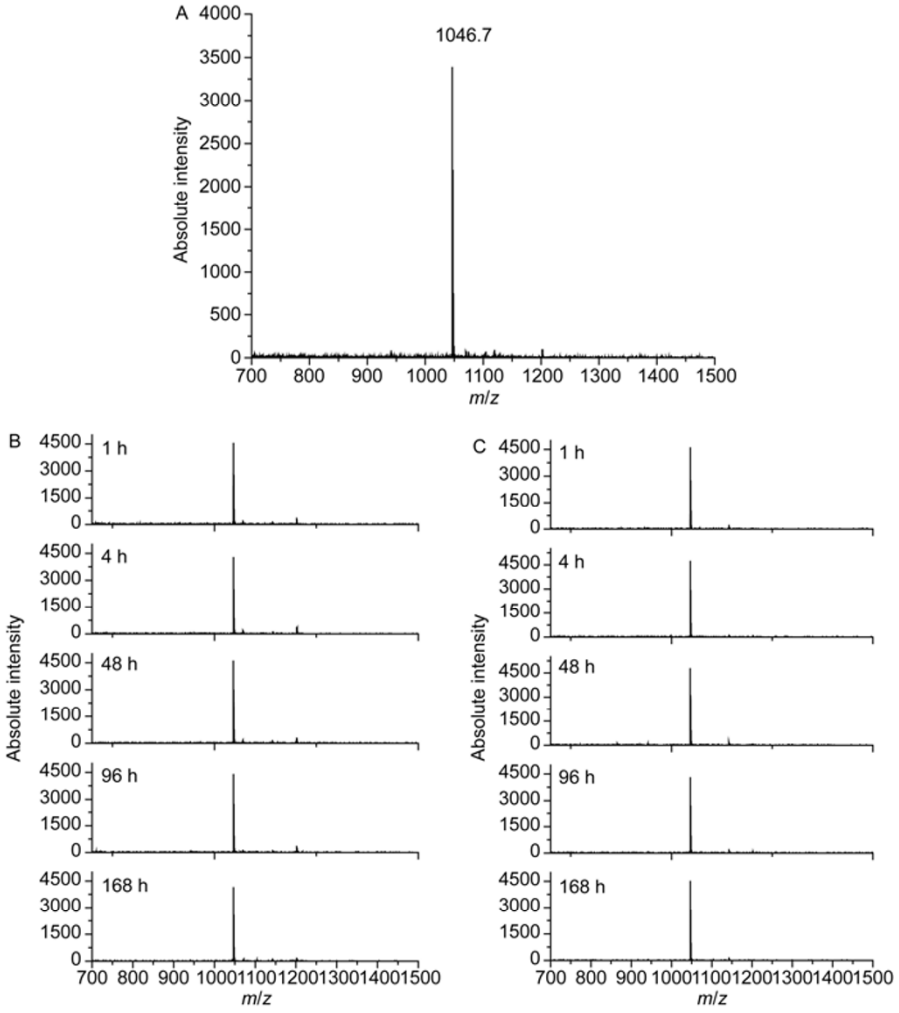

Figure 2 MALDI-TOF mass spectra of RI-7 derivatized by BAPI (A), and its stability in water containing 0.1% TFA (B) or 50% acetonitrile/water containing 0.1% TFA (C).

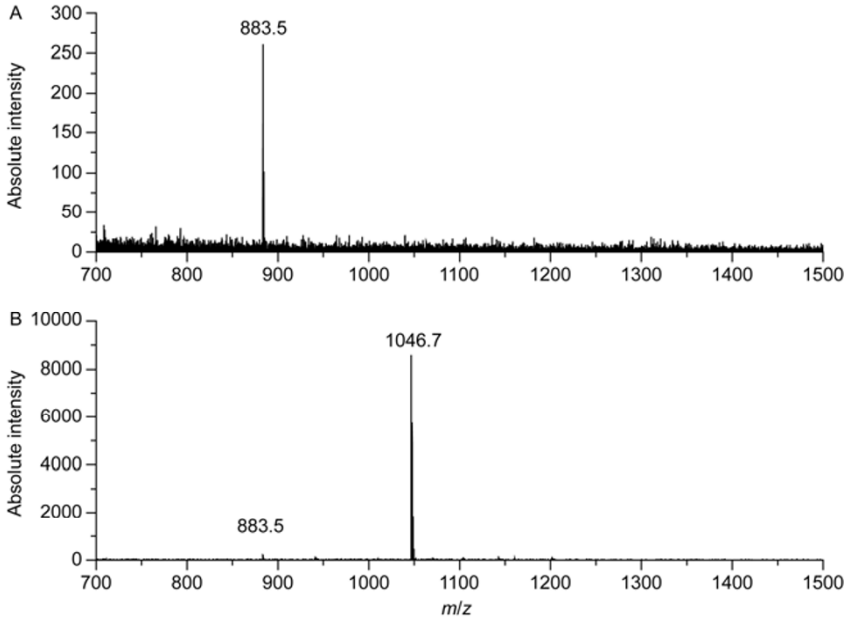

Figure 3 MALDI-TOF mass spectra of equimolar mixtures of native RI-7 with the blank solution (A) and BAPI derivatized RI-7 (B).

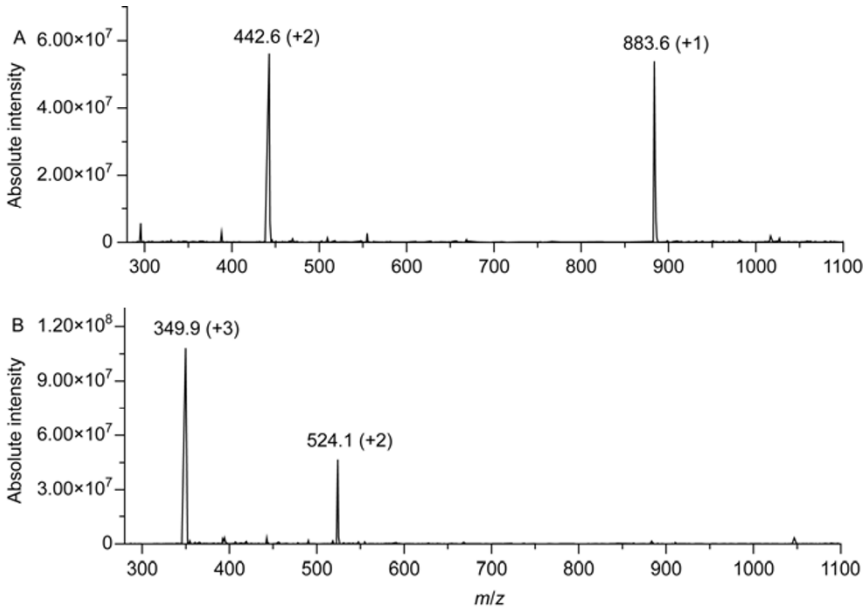

Figure 4 ESI MS analysis of native (A) and BAPI derivatized RI-7 (B).

The results suggest the potential merits of BAPI as a candidate derivatization reagent for high sensitivity peptide analysis by MS.

This work was supported by National Basic Research Program of China (2012CB910604), National Natural Science Foundation of China (21205027, 21005079, 20935004), Analytical Method Innovation Program of Ministry of Science and Technology of China (2010IM030500), and Natural Science Foundation of Hebei Province (B2012201095).

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