Talanta 126 (2014) 91–102

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Review

Mass spectrometry-based tag and its application to high efficient peptide analysis – A review

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ARTICLE INFO

Article history:
Received 23 December 2013
Received in revised form
27 February 2014
Accepted 10 March 2014
Available online 27 March 2014

Keywords:
Tag
High efficient analysis
Peptide
Mass spectrometry

ABSTRACT

Chemical derivatization is a very promising technique for improving analysis of peptides by mass spectrometry (MS). Thereinto, development of novel tags compatible with MS and/or MS/MS has always been the focus point of study. In this review, the recent reported tags for derivatization of thiol groups of cysteine, carboxyl groups, and amino groups on peptides as well as peptides with post-translational modifications (PTMs) are summarized. Moreover, the tags used for derivatization of glycans or oligosaccharides released from glycoproteins are also reviewed.

Contents

1. Introduction

Since the completion of human genome sequencing, the human genome project has been entering into the post-genomic era [1,2], of which proteomics, as an infant research paradigm, has been paid much more attention [3]. The major approach used for proteome research is the bottom-up or shotgun strategy. In a typical procedure, proteins are firstly digested into peptides,

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http://dx.doi.org/10.1016/j.talanta.2014.03.012
0039-9140/

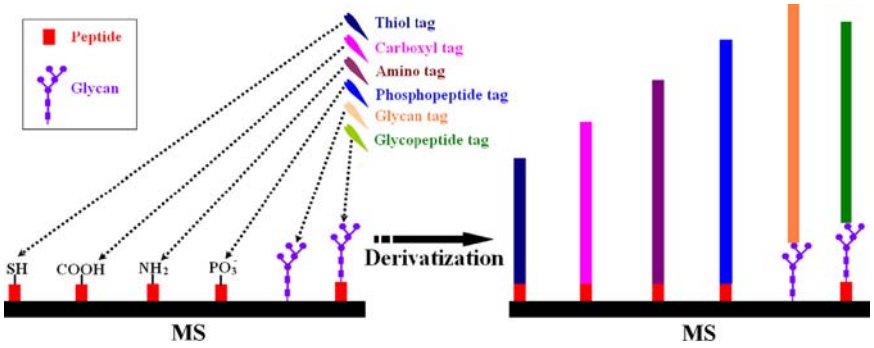


Fig. 1. Schematic diagram of the review.

followed by multi-dimensional high performance liquid chromatography (HPLC) for peptides separation and tandem mass spectrometry (MS/MS) for peptides sequencing [4–6]. Obviously, MS-based technique has become an important tool in proteome research. Especially, with the emergence and wide usefulness of soft ionization techniques, such as matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI), MS has been evolving into an indispensable technology for qualitative and quantitative analysis of proteins or peptides [7,8].

However, many proteins, such as the drug targets or biomarkers, are often present in low concentration in real protein samples [9,10]. Thus, it is very difficult to detect these proteins in a diverse "sea" of complex proteins. Moreover, the ionization efficiency of peptides in MS is often structure-dependent. Thus, many peptides, such as phosphopeptides and glycopeptides, are difficult to ionize in MS [11,12]. Therefore, improving analysis of these peptides is crucial for further in-depth proteome research.

Chemical derivatization [13–16] is a very promising method for improvement of ionization and detection of these samples. It could even retrospect to the early 1960s when trifluoroacetic anhydride and methanol were used for rapid acylation and esterification of naturally occurring amino acids so as to improve the determination of amino acid ratios in peptides as well as qualitative determination of amino acids in proteins [17]. In the past few decades, numerous tags have been developed and further used for derivatization of thiols groups of cysteine, carboxyl groups, and amino groups on peptides as well as peptides with post-translational modifications (PTMs). In this review, these tags are systematically summarized and further classified according to the target reactive groups on peptides. Furthermore, tags developed for labeling of glycans or oligosaccharides released from glycoproteins are also introduced. The representative scheme of this review is shown in Fig. 1.

2. Characteristics of the developed tags

In the late-1990s, Krause et al. [18] analyzed the mycobacteria proteome by combining with two-dimensional electrophoresis (2-DE) for proteins separation and MALDI- time-of-flight (TOF) MS for peptides identification. Interestingly, 94% of the most sensitive peaks were found to be arginine-containing peptides. It could be attributed to the excellent basicity of guanidine groups of the arginine side chain, which could promote peptide ionization in liquid and/or gas phase, yielding high MS signal. Pashkova and Chiappetta [19,20] further provided evidence that hydrophobic peptides are more likely to co-crystallize with the hydrophobic matrix, allowing more sensitive identification by MALDI source. In ESI–MS, peptides with strong basicity and high hydrophobicity are also more inclined to protonate and ionize during the desolvation process, achieving high-efficiency peptide analysis [21]. Obviously, basicity and hydrophobicity are crucial factors for peptide analysis by MS.

Thus, most of the tags were designed with the following structures: (1) guanidine group, tertiary amines, or quaternary ammonium moieties with high basicity; (2) hydrophobic chains or aromatic groups with strong hydrophobicity; and (3) reactive groups for targeted peptide labeling. In fact, many tags were designed with all of the above-mentioned characteristics.

3. Derivatization of unmodified peptides

3.1. Thiol group of cysteine

Cysteine is an attractive target for peptide labeling due to its high reactivity, low abundance, and universal distribution in a variety of proteomes [22]. Many hydrophobic tags and quaternary ammonium tags were reported for derivatization of thiol group, as summarized in Table 1.

Ueberheide et al. [23] firstly applied N,N-dimethyl-2-chloroethylamine to derivatize thiol groups of toxins so as to increase the charge state of these peptides prior to electron-transfer dissociation (ETD) MS/MS analysis. Totally 31 intact individual toxins were successfully sequenced from crude venom sample from Conus textile.

Li et al. [24] synthesized a novel maleimidyl-containing tag, 1-[3-(4-maleimidylphenoxy)propyl]trimethylammonium bromide, for labeling of cysteine-containing peptides. The reaction was allowed to proceed for 2 h at 37 °C with a derivatization yield close to 100%. Furthermore, the ionization efficiency increased over 100-fold for peptides with less polar residues via MALDI-TOF MS analysis, while the ionization efficiency for peptides with more polar residues could increase only 3–5-fold.

(3-Acrylamidopropyl)trimethylammonium chloride (APTA) was initially used for derivatization and enrichment of cysteine-containing peptides by Ren et al. prior to MS analysis [25]. Vasicek and Brodbelt [26] further evaluated the effect of APTA derivatization on the ETD efficiency of peptides. The results indicated that both the charge states and ETD dissociation efficiency for all the peptides simultaneously increased, outperforming the commonly used tag iodoacetamide (IAA) and N,N-dimethyl-2-chloro-ethylamine [23]. The method was further used for analysis of tryptic digest of bovine serum albumin (BSA), and the SEQUEST score was increased to 3700 from 582 via derivatization. The main drawback of APTA derivatization was relatively low labeling efficiency. It was estimated to be about 70% by comparing the summed area of APTA derivatized products to the summered area of both derivatized products and the native peptides.

In fact, most of the reported tags were functionalized with iodoacetamide group. Muddiaman's group developed a variety of these types of tags to increase the MS response of cysteine-containing peptides. 2-Iodo-N-octylacetamide [27] was firstly

Table 1
Tags for derivatization of thiol group of cysteines.

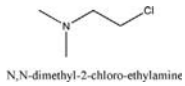
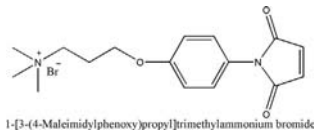
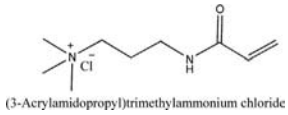
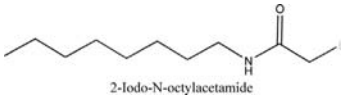
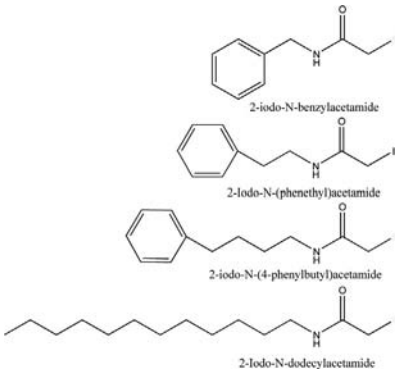
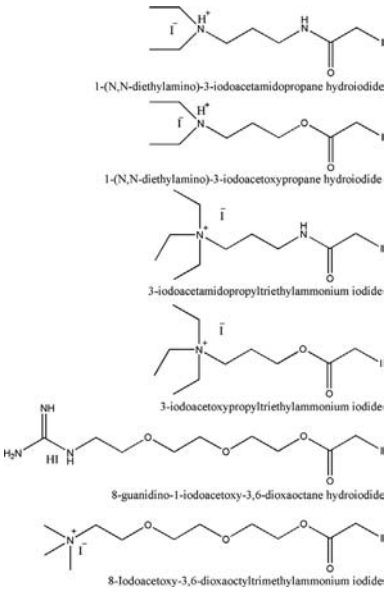
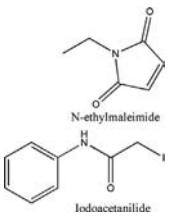
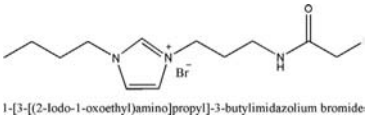
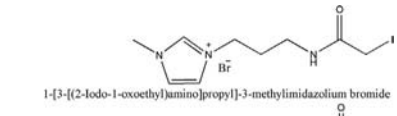
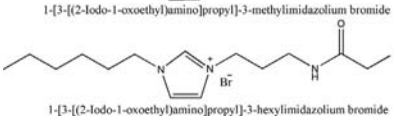
Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
 N,N-dimethyl-2-chloro-ethylamine	Toxin	ESI	LTQ/LTQ-Orbitrap	–	–	[23]
 1-[3-(4-Maleimidylphenoxy)propyl]trimethylammonium bromide	Model peptide	MALDI	TOF	Over 100-fold for less polar peptides/3–5-fold for polar peptides	–	[24]
 (3-Acrylamidopropyl)trimethylammonium chloride	Model peptide	ESI	LTQ	–	BSA	[26]
 2-Iodo-N-octylacetamide	Model peptide	ESI	LTQ-FT	2–3-fold	–	[27]
 2-Iodo-N-benzylacetamide 2-Iodo-N-(phenethyl)acetamide 2-Iodo-N-(4-phenylbutyl)acetamide 2-Iodo-N-dodecylacetamide	Model peptide	ESI	FT-ICR	Up to 2441-fold	–	[28]
 1-(N,N-diethylamino)-3-iodoacetamidopropane hydroiodide 1-(N,N-diethylamino)-3-iodoacetoxypropane hydroiodide 3-iodoacetamidopropyltriethylammonium iodide 3-iodoacetoxypropyltriethylammonium iodide 8-guanidino-1-iodoacetoxy-3,6-dioxaoctane hydroiodide 8-Iodoacetoxy-3,6-dioxaoctyltrimethylammonium iodide	Model peptide	MALDI	TOF	16.5–222.5-fold	–	[30]
 N-ethylmaleimide Iodoacetanilide	Model peptide	ESI	–	5–20-fold	–	[31]

Table 1 (*continued*)

Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
 1-[3-[(2-Iodo-1-oxoethyl)amino]propyl]-3-butylimidazolium bromide	Model peptide	MALDI	TOF	62–114-fold	BSA	[33]
 1-[3-[(2-Iodo-1-oxoethyl)amino]propyl]-3-methylimidazolium bromide  1-[3-[(2-Iodo-1-oxoethyl)amino]propyl]-3-hexylimidazolium bromide	Model peptide	MALDI	TOF	27–192-fold	BSA	[34]

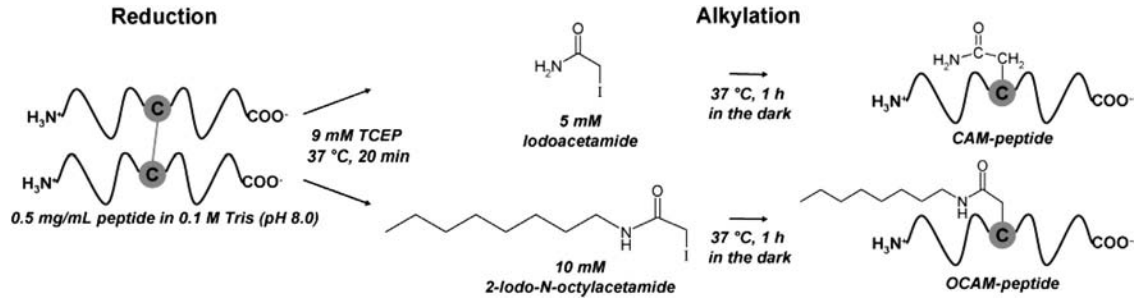


Fig. 2. Strategy for derivatization of cysteine-containing peptides with iodoacetamide and 2-iodo-N-octylacetamide. Reprinted from [27], copyright 2007, with permission of American Chemical Society.

synthesized for derivatization of cysteine-containing peptides, as shown in Fig. 2; a 3-fold and 2-fold improvement in MS response was achieved for model peptide laminin nonapeptide and B-type natriuretic peptide-32, respectively. Furthermore, no fragment and neutral loss were observed from the collision induced dissociation (CID) MS/MS spectra. Thus, the labeling reaction was suitable for the peptide sequencing. Later, several hydrophobic alkyl tags, including 2-iodo-N-benzylacetamide, 2-iodo-N-(phenethyl)acetamide, 2-iodo-N-(4-phenylbutyl)acetamide, and 2-iodo-N-dodecylacetamide, [28] were further synthesized for derivatization of cysteine-containing peptides. The MS response of model peptide CYFQNCPRG derivatized via 2-iodo-N-dodecylacetamide was 2441 times higher than that labeled by commercially available IAA tag. Moreover, the tag was proved to be more efficient than the previous reported tag 2-iodo-N-octylacetamide [27]. These tags were further used for derivatization of a low abundance cardiac biomarker, B-type Natriuretic peptide, and the limit of detection (LOD) was decreased about 3.5-fold via 2-iodo-N-octylacetamide derivatization [29].

Shimada et al. [30] synthesized several new tags containing of tertiary amino, quaternary ammonium, or guanidino groups, of which tags designed with quaternary ammonium moiety were proved to be more efficient than that with the tertiary amine or guanidino groups via MALDI-TOF MS analysis. For example, the MS response of model peptides insulin alpha, insulin beta chain, NC4 CLAC-P, and S26C beta-amyloid 17–40 derivatized by 8-iodoacetoxy-3,6-dioxaoctyltrimethylammonium iodide could respectively increase 222.5, 58.3, 33.5, and 16.5-fold, compared with those labeled by IAA.

Zabet-Moghaddam et al. [31,32] reported derivatization of cysteine-containing peptides with iodoacetanilide and N-ethylmaleimide. The ionization efficiency of model peptides PEP60, PEP13, and PEP31 was increased about 5–20-fold compared with those labeled by IAA via ESI–MS. The authors further applied iodoacetanilide for derivatization of tryptic digest of BSA, and the sequence coverage was increased to 33% from 24% via MALDI-TOF MS, while the score could be increased to 255 from 203, achieving high confident identification.

Qiao et al. [33] further reported a novel imidazolium-based aromatic quaternary ammonium tag, 1-[3-[(2-iodo-1-oxoethyl) amino]propyl]-3-butylimidazolium bromide (IPBI), for efficient analysis of cysteine-containing peptides. The ionization efficiency of model peptides CDPGYIGSR and ALVCEQEAR could respectively increase about 100 and 114-fold compared with the native cognates via MALDI-TOF MS analysis. The tag has also been proved to be more efficient than the previous reported tag iodoacetanilide [31]. The authors further synthesized and compared the effect of 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-3-methylimidazolium bromide (IPMI), IPBI, and 1-[3-[(2-iodo-1-oxoethyl)amino]propyl]-3-hexylimidazolium bromide (IPHI) derivatization on the MS performance of cysteine-containing peptides, of which IPHI, possessing the strongest hydrophobicity, indicated the most obvious ionization efficiency improvement via MALDI-TOF MS [34].

The labeling reaction of the tags containing iodoacetamide group often performed within 2 h with a 100% or nearly 100% derivatization yield. Some of the tags, such as IPMI, IPBI, and IPHI, indicated with excellent stability. For example, peptides derivatized by IPHI could stabilize in the reaction buffer at least one week.

3.2. Carboxyl group

In the past few years, the derivatization of carboxyl group on peptides has been paid much attention. Especially, many tags were designed suitable for ETD induced fragmentation. All these tags have been summarized in Table 2.

Table 2
Tags for derivatization of carboxyl groups.

Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
1-(2-Pyrimidyl)piperazine	Model peptide Model phosphopeptide	MALDI	TOF	10–40-fold 50–101-fold	BSA and a phosphopeptide mixture	[35]
1-(2-Pyridyl)piperazine	Model peptide	MALDI	TOF	12.2–14.1-fold	Rat brain protein fractions	[37]
2, 4-dimethoxy-6-piperazin-1-yl pyrimidine	Model peptide	MALDI	TOF	20–25-fold	Lysozyme, β-casein and BSA	[38]
1-(3-Aminopropyl)-3-butylimidazolium bromide	Model peptide	MALDI	TOF	42.2-fold	–	[39]
2-Nitrophenylhydrazine	Model peptide	ESI	LTQ	1.2–15.7-fold	–	[40]
(2Aminoethyl)trimethylammonium Girard Reagent T	Model peptide	ESI	LTQ	–	Cytochrome c	[41]
C3-methylTert C5-methylTert C4-ethylTert C5-isopropylTert	BSA	ESI	LTQ-Orbitrap	–	Yeast ribosomal proteins	[42]

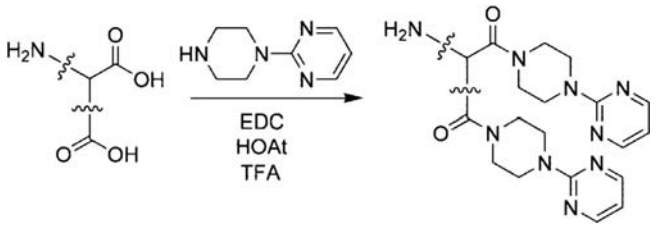


Fig. 3. Strategy for derivatization of carboxyl groups on peptides with 1-(2-pyrimidyl)piperazine. Reprinted from [35], copyright 2008, with permission of American Chemical Society.

Xu et al. [35] developed the method to derivatize carboxyl groups on peptides with 1-(2-pyrimidyl)piperazine, as shown in Fig.3. The authors found the tag was especially suitable for analysis of phosphopeptides, and the ionization efficiency of four model phosphopeptides increased by 50–101-fold via MALDI-TOF MS. The same group further proved that 1-(2-pyrimidyl) piperazine derivatization could simultaneously increase the charge states and improve the retention of hydrophilic phosphopeptides in RPLC, showing great advantages for peptide sequencing via ETD MS/MS [36]. The main advantage of 1-(2-pyrimidyl)piperazine derivatization was rapid reaction time and high derivatization yield. With the addition of a certain amount of trifluoroacetic acid, a nearly 100% yield was achieved within a few seconds reaction at room temperature. Moreover, the peptide derivatives could stabilize in water or TFA solution at least for two days. However, the reaction is slightly pH sensitive. Thus, the pH value should be strictly controlled during the derivatization procedure. Qiao et al. [37] expanded and compared the effect of a series of piperazine derivatives on the ionization efficiency of peptides, of which 1-(2-pyridyl)piperazine and 1-(2-pyrimidyl)piperazine, respectively with the highest hydrophobicity and gas-phase hydrogenation capacity, exhibited the obvious improvement of ionization efficiency by MALDI-TOF MS. Leng et al. [38] further synthesized novel piperazine derivatives, 2,4-dimethoxy-6-piperazin-1-yl pyrimidine (DMPP) and isotope deuterium (d) introduced tag d_6-DMPP for quantitative determination of peptides with improved ionization efficiency via MALDI-TOF MS.

Qiao et al. [39] further synthesized and evaluated the derivatization of carboxyl group on peptides with 1-(3-aminopropyl)-3-butylimidazolium bromide. With model peptide RVYVHPI as the sample, a nearly 100% derivatization yield was achieved within 10 s reaction at room temperature. Furthermore, the ionization efficiency of the derivatized peptide increased over 42-fold via MALDI-TOF MS, outperforming commercially available piperazine derivatives [37].

2-Nitrophenylhydrazine was reported by Zhang et al. [40] for derivatization of carboxyl groups on peptides. It could simultaneously improve the hydrophobicity and increase the retention time for all the test peptides, resulting in high MS response for some of the peptides. Although 2-Nitrophenylhydrazine derivatization could be completed within 30 s, the labeling efficiency was slightly low, estimated to be only 80% or higher.

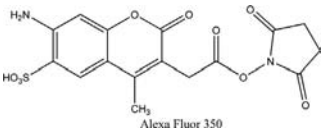
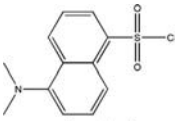
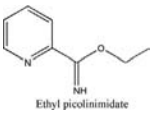
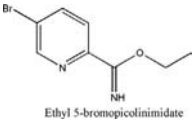
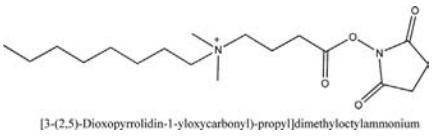
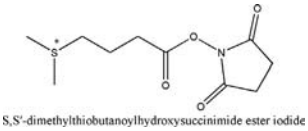
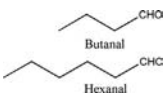
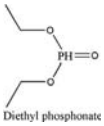
Ko and Brodbelt [41] compared the effect of benzylamine (BA), 1-benzylpiperazine (BZP), carboxymethyl trimethylammonium chloride hydrazide (GT), and (2-aminoethyl)trimethylammonium chloride hydrochloride (AETMA) derivatization on the ETD MS/MS performance of peptides. Peptides derivatized by the fixed charge tags GT or AETMA showed the highest ETD efficiencies and great number of diagnostic fragment ions. For model peptides, the ETD efficiencies could be increased to 22% from 17% via AETMA or GT derivatization. The authors further applied the tag AETMA for derivatization of tryptic digest of cytochrome c; the total number of identified peptides could be increased to 49 from 26 while the Xcorr score could be increased to 71 ± 10 from 40 ± 8. The main drawback of the derivatization method was that it was too time-consuming (usually up to 1 h), and the derivatization yield was estimated only as 70% or higher.

Frey et al. [42] reported the derivatization of carboxyl groups on peptides with a series of tertiary or quaternary amine tags. With tryptic digest of BSA as the sample, alkyl tertiary amines were found to be the most efficient labeling reagents; the sequence coverage could be increased to 70% from 43% via ETD MS/MS. The authors further applied the tag C3-methylTert for derivatization of tryptic digest of yeast ribosomal proteins. The sequence coverage of the identified proteins could be increased over 50%, and doubled via peptide spectral matches. However, it must be noted that, although an average derivatization yield of 99% was achieved, the labeling process was often too time-consuming. A step of pre-methylation of amines was needed before the derivatization reaction.

3.3. Amino group

The tags reported for the derivatization of amino groups on peptides were summarized in Table 3. Early researches have reported the derivatization of peptides with commercially available fluorescent tags. For example, Pashkova et al. [19,43] exploited to derivatize peptides with coumarin derivatives. The labeling reaction was allowed to proceed for 40 min at room temperature. For model peptides, a 5–15-fold increment in intensity was achieved via Alexa Fluor 350 derivatization and MALDI-TOF MS analysis. The method was further used for analysis of tryptic digests of SCX fractions of *E. coli* lysate, and the total number identification of proteins was increased about 30% via LC–MALDI-TOF/TOF MS. Chiappetta et al. [20] further applied

Table 3
Tags for derivatization of amino groups.

Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
 Alexa Fluor 350	Model peptide	MALDI	TOF	5–15-fold	SCX fractions of *E. coli* lysate	[19,43]
 Dansyl chloride	BSA	MALDI	TOF	–	*E. coli* proteins	[20]
 Ethyl picolinimidate	Model phosphopeptide	MALDI	TOF	Over 100-fold	–	[45]
 Ethyl 5-bromopicolinimidate	Model peptide	MALDI	TOF	10-fold	Myoglobin and hemoglobin	[46]
 [3-(2,5)-Dioxopyrrolidin-1-yloxycarbonyl)-propyl]dimethyloctylammonium	Model peptide	ESI	–	10-fold	Transferrin	[21]
 S,S'-dimethylthiobutanoylhydroxysuccinimide ester iodide	Model phosphopeptide	ESI	LTQ	2.5-fold	–	[47]
 Butanal Hexanal	Model peptide	ESI	Q-TOF/LCQ	Up to 34-fold	Cytochrome c, β-lactoglobulin A, and BSA	[48]
 Diethyl phosphonate	Model peptide	ESI	Q-TOF	–	BSA	[49]

dansyl chloride to derivatize the primary amino groups on peptides, and the ionization efficiency of the most hydrophobic peptides could be obviously improved.

Ethyl picolinimidate [44] was developed by Kim et al. [45] for picolinamidination the amino group of peptides. For model phosphopeptides, the ionization efficiency was increased at least 100-fold via MALDI-TOF MS. Furthermore, enhanced b ion series were observed from the MS/MS spectra, which especially benefit for deduction of the sequence of phosphopeptides and identification of the phosphorylation sites. However, the labeling reaction was slightly time-consuming, and it was performed overnight at room temperature. The same group [46] further synthesized a Br element embed tag, ethyl 5-bromopicolinimidate, to derivatize N-terminal amino group of peptides. For model peptides ASHLGLAR, AEQpSLKDVNK, and AEDDVEDY, the ionization efficiency was roughly increased about 10-fold via MALDI-TOF MS. Interestingly, a series of b-ions with Br signature were observed from the MS/MS spectra, enabling direct N-terminal sequencing of proteins.

Mirzaei and Regnier [21] synthesized a novel labeling reagent, [3-(2,5)-dioxopyrrolidin-1-yloxycarbonyl)-propyl]dimethylocty-lammonium, that could simultaneously introduce a permanent positive charge and hydrophobic moiety to the labeled peptides within 2 h reaction at room temperature. The ionization efficiency of the derivatized peptides could be increased about 10-fold via ESI–MS. Furthermore, the method was proved to be especially efficient for peptides with the molecular weight less than 500 Da.

Lu et al. [47] synthesized a novel fixed charge sulfonium ion reagent, S,S'-dimethylthiobutanoylhydroxysuccinimide ester iodide, for derivatization of the amino groups on peptides. The reaction was allowed to proceed for 30 min at room temperature in the dark, and the ionization efficiency of 50 model phosphopeptides could be increased average 2.5-fold via ESI–MS. Furthermore, isotope deuterium introduced tags were simultaneously synthesized. Thus, differential quantitative analysis of phosphopeptides was achieved with enhanced quantitative capability in both ETD MS/MS and CID MS/MS.

Kulevich et al. [48] used aldehyde-based tag butanal and hexanal to derivatize the amino groups on peptides, as shown in Fig. 4. The ionization efficiency of model peptide GGYR derivatized by hexanal was increased about 34-fold via ESI–MS. The method was further used for analysis of tryptic digests of cytochrome c, β-lactoglobulin A, BSA, and the combined sequence coverage could be respectively increased to 63%, 67%, and 60% from 48%, 64%, and 53% by simultaneously analyzing both butanal derivatized and the native protein digests. The main drawback of aldehyde-based derivatization reaction was relatively long labeling time. The reaction proceeded overnight at room temperature.

Zhang et al. [49] developed an effective method for peptide sequencing based on phosphorylation labeling strategy. The derivatization reaction was completed within 13 min at room temperature. Once peptides were labeled by diethyl phosphonate, the signal intensity of both the a_1 ion and b series ions increased in the ESI–MS/MS spectra, providing much more useful information for peptide sequencing. Gao et al. [50] further used diisopropyl phosphate for derivatization of amino acids and small peptides with improved ionization efficiency and decreased ion suppression effects by MALDI-TOF MS.

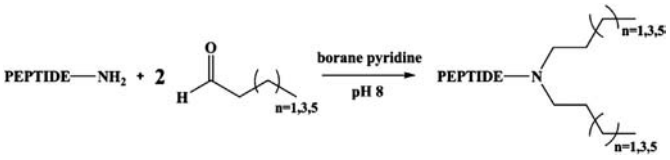
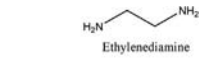
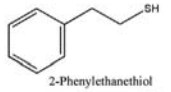
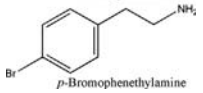
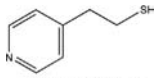
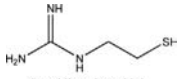
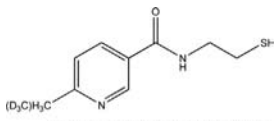


Fig. 4. Strategy for derivatization of primary amines on peptides with aldehydes of varying lengths. Reprinted from [48], copyright 2010, with permission of American Chemical Society.

4. Derivatization of peptides with PTMs

Proteins with PTMs are often present in low concentration and difficult to ionize in MS, especially with the ion suppression effect by the vast majority of non-modified peptides. However, these proteins always play important roles in biological processes, and even many of them have been proved to be the therapeutic targets and biomarkers for cancer, such as glycoprotein prostate-specific

Table 4
Tags for derivatization of phosphopeptides.

Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
 Ethylenediamine	Model peptide	MALDI	TOF	4.4–11.6-fold	Ovalbumin	[53]
 2-Phenylethanethiol						
 *p*-Bromophenethylamine						
 Mercaptoethylpyridine	α-Casein	MALDI	TOF	1–100-fold	Human protein kinase Akt1	[54]
 Guanidinoethanethiol	α-Casein	MALDI	TOF	Up 22-fold	–	[55]
 N-(2-mercaptoethyl)-6-methylnicotinamide	Model peptide	MALDI	TOF	5-fold	Ovalbumin	[56]

antigen for prostate cancer [51,52]. Thus, the development of tags suitable for efficient analysis of proteins with PTMs is crucial for further in-depth research of these proteins. Up till now, tags designed for derivatization of phosphopeptides, glycopeptides as well as the glycans or oligosaccharides released from glycoproteins have been reported.

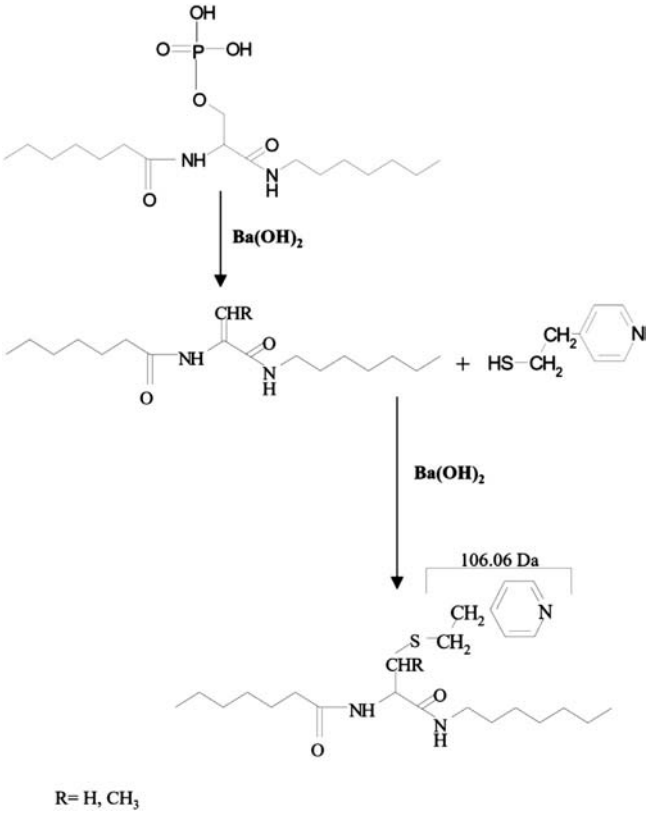


Fig. 5. Strategy for derivatization of serine/threonine phosphopeptides with mercaptoethylpyridine. Reprinted from [54], copyright 2006, with permission of John Wiley and Sons.

4.1. Phosphopeptides

The major phosphopeptide derivatization method is based on beta-elimination/Michael addition reaction. As a result, the phosphate groups of phosphoserines or phosphothreonines could be replaced by the tagging reagents, achieving phosphopeptide labeling. These tags were summarized in Table 4.

Klemm et al. [53] reported the derivatization of phosphopeptides with a series of N- and S-nucleophiles, of which peptides labeled by ethylenediamine, 2-phenylethanethiol, or *p*-bromophenethylamine showed clear improvement of ionization efficiency. For example, the tag 2-phenylethanethiol was used for derivatization of ovalbumin and human Stat1, and a signal increment of 3.4–38-fold was achieved for the corresponding phosphopeptides via MALDI-TOF MS.

Arrigoni et al. [54] reported using mercaptoethylpyridine to derivatize phosphopeptides, as shown in Fig. 5. For model protein α-casein, the average signal intensity for the derivatized peptides increased 1–100-fold compared with the singly phosphorylated phosphopeptides via MALDI-TOF MS. Furthermore, diagnostic fragment at m/z 106.06 could be observed from the CID MS/MS spectra, which could be potentially used for precursor ion scanning so as to selectively detect modified peptides in complex mixtures. The method was further applied for analysis of human protein kinase Akt1 using an in-gel derivatization procedure, and as yet unidentified sites, protein kinase CK2 phosphorylation sites, were proved with experimental proofs.

Ahn et al. [55] exploited for derivatization of phosphopeptides with guanidinoethanethiol (GET). Since the phosphate group carrying the negative charge was replaced by guanidinoethylcysteine containing a guanidine moiety, the gas-phase basicity of the derivatized peptides was largely increased. Thus, the number of recognized phophopeptides from the tryptic digest of alpha-casein was increased to 9 from 5 via MALDI-TOF MS analysis. Especially, even though 10 fmol of the tryptic digest of α-casein was deposited on the target, GET labeled peptides could still be recognized with reasonable signal-to-noise (S/N) ratios. Moreover, the MS/MS efficiency of the derivatized peptides could also be improved.

Tsumoto et al. [56] synthesized novel tags, N-(2-mercaptoethyl)-6-methylnicotinamide (MEMN) and d_3-labeled MEMN for derivatization of phosphopeptides; both improved ionization

Table 5
Tags for derivatization of glycans or oligosaccharides.

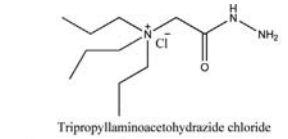
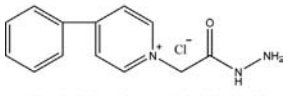
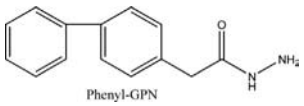
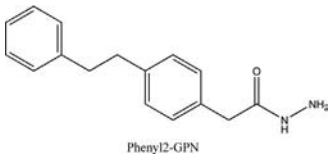
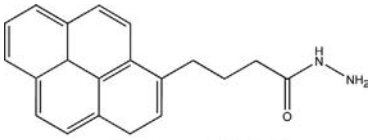
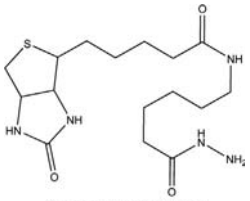
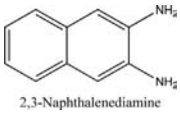
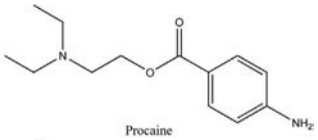
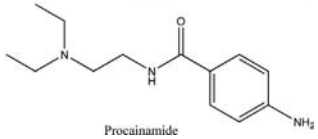
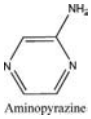
Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
 Tripropyllaminoacetohydrazide chloride	Model glycan	ESI	LTQ-FT-ICR	5-fold	–	[59]
 Phenylpyridineaminoacetohydrazide chloride						
 Phenyl-GPN	Model glycan	ESI	LTQ-FT-ICR	18-fold	–	[60]
 Phenyl2-GPN	Model glycan	ESI	LTQ-FT-ICR	–	Plasma proteins	[61]

Table 5 (*continued*)

Tag	Analyte	Ion source	Analyzer	Sensitivity	Sample	Reference
 Pyrene butanoic acid hydrazide	Model glycan	MALDI	TOF	33-fold	Human plasma	[63]
 Biotinamidocaproyl hydrazide	Model oligosaccharide	ESI/MALDI	Ion-Trap/TOF	1–10-fold	Ovalbumin	[66]
 2,3-Naphthalenediamine	Model oligosaccharide	MALDI	TOF	< 1 pmol	–	[67]
 Procaine  Procainamide	Model oligosaccharide	MALDI	TOF	–	–	[68]
 Aminopyrazine	Model oligosaccharide	MALDI	TOF	10-fold	Ribonuclease B and ovalbumin	[69]

efficiency and relative quantitative research could be achieved. For example, the ionization efficiency of model peptide TNApSYSPRAK was increased approximate 5-fold via MALDI-TOF MS analysis, compared with the previous reported tag mercaptoethylpyridine [54]. Furthermore, as the quality of peptide increased from 1.25 pmol to 12.5 pmol, peptides derivatized by d_3-labeled MEMN or MEMN indicated good quantitative results, with a correlation coefficient of 0.9977.

Beta-elimination/Michael addition-based labeling reaction often performed at room temperature or 37 °C for 2 h. The major drawback of the method is that the analysis of phosphotyrosine containing peptides was failed. Furthermore, the presence of O-linked glycopeptides could disturb the identification of phosphopeptides.

4.2. Glycans or oligosaccharides

Protein glycosylation is one of the most significant PTMs in nature, and altered glycosylation has been reported to associate with a variety of diseases [57]. Thus, it is very importance to understand the structure of glycans or oligosaccharides and how they are altered in a disease state. The main problem for analysis of native glycans or oligosaccharides released from glycoproteins is the poor ionization efficiency in MS. Thus, many tags have been developed for derivatization of glycans or oligosaccharides to improve their analysis by MS, as summarized in Table 5.

GT was initially developed by Naven and Harvey [58] to derivatize and improve the analysis of oligosaccharides in MS. The labeling reaction was allowed to proceed for 3 h at 75 °C with high derivatization yield (95–100%). For maltoheptaose, the ionization efficiency could be increased 10-fold and the LOD could be down to 50 fmol via MALDI MS analysis. The method was further used for derivatization of high-mannose oligosaccharides released from bovine ribonuclease B, and a 10-fold increment in signal intensity was achieved compared with the native counterparts. Furthermore, in ESI–MS, improved ionization efficiency was also observed via derivatization.

Muddiman's group did a lot of excellent work on the development of novel hydrazide-containing tags for derivatization of glycans. The labeling efficiency by these tags was usually over 95% within 3 h reaction. Tripropyllaminoacetohydrazide chloride and phenylpyridineaminoacetohydrazide chloride [59] were firstly synthesized, and the ESI–MS response of derivatized model glycans could increase about 5-fold, outperforming commercial GT reagent derivatization [58]. Muddiman et al. [60] further compared the difference of tags with a permanent positive charge or hydrophobic moieties on the ESI response of glycans. The results indicated that glycans labeled with increasingly hydrophobic tags outperformed those derivatized by tags with a positive charge. The probable reason was that tags with a permanent positive charge could induce the labeled glycans to be more likely to solvate in the ESI droplet, resulting in decreased MS response. Thus, several of neutral, hydrophobic tags were further designed and synthesized for derivatization of N-linked glycans, of which phenyl2-GPN, designed with strongest hydrophobicity, was the

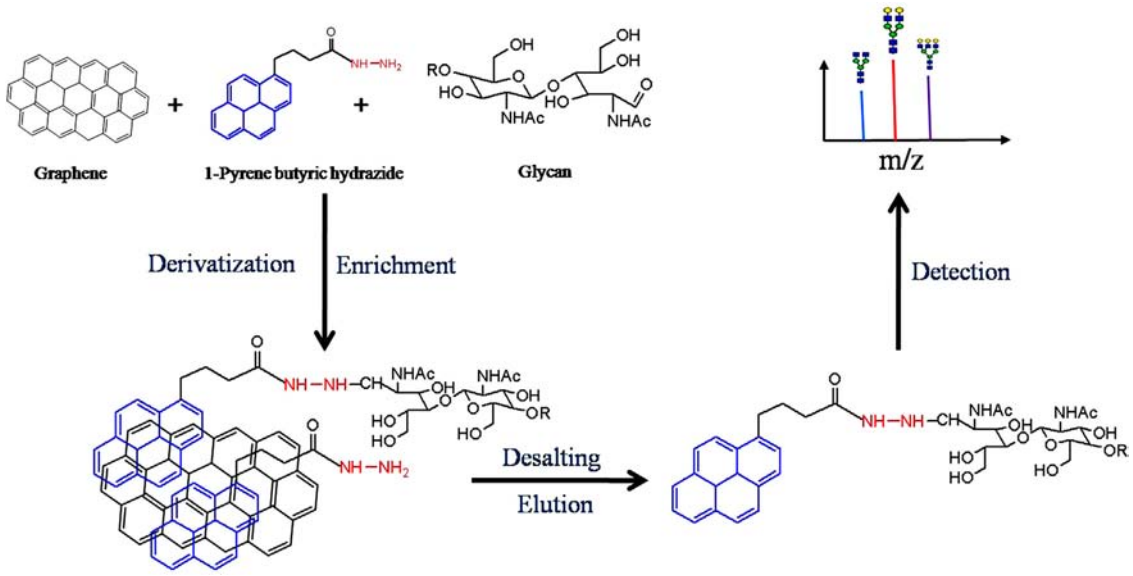
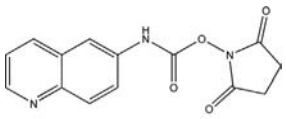
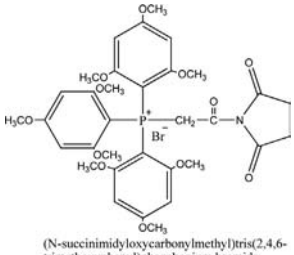
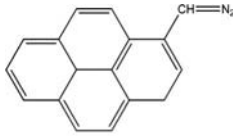


Fig. 6. Schematic overview of free rGO based "one-step" method for glycan enrichment, derivatization and MS analysis. Reprinted from [63], copyright 2013, with permission of Elsevier.

Table 6
Tags for derivatization of glycopeptides.

Tag	Analyte	Ion source	Analyzer	LOD	Reference
 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate	Antithrombin, ovalbumin, and α1-acid-glycoprotein	MALDI/ESI	TOF/Ion-Trap	low fmol	[70]
 (N-succinimidyloxycarbonylmethyl)tris(2,4,6-trimethoxyphenyl)phosphonium bromide	Model glycopeptide and phosphopeptide	ESI	FT-ICR	–	[71]
 1-pyrenyldiazomethane	Prostate specific antigen	MALDI	TOF	1 ng	[72]

most efficient tag for glycan analysis via ESI–MS [61]. In their most recent report, ^{13}C stable isotope labeled tag phenyl2-GPN [62] was synthesized and further exploited for relative quantification of N-linked glycans via ESI–MS.

Bai et al. [63] developed the first one-step method for simultaneous enrichment and derivatization of glycans using reduced graphene oxide as nanoreactors and 1-pyrenebutyric hydrazide [64,65] for glycan capture and labeling, as shown in Fig. 6. The one-step procedure was allowed to proceed for 1 h at 90 °C with a nearly 100% derivatization yield. With the developed method, using only 10 μL of human plasma, 48 possible N-glycoforms were readily identified from human plasma via MALDI-TOF MS.

Kapková [66] exploited to derivatize oligosaccharides with biotinamidocaproyl hydrazide. The labeling reaction was performed at 90 °C for 1 h, and the ESI–MS response of derivatized lactose increased about an order of a magnitude compared with the native cognate. The method was further used for analysis of N-linked glycans released from hen ovalbumin, and improved MALDI-TOF MS analysis was also achieved.

Lin et al. [67] developed the method to derivatize oligosaccharides with 2,3-naphthalenediamine. By iodine-promoted oxidative condensation, the oligosaccharides could be easily converted into the corresponding naphthimidazole derivatives. With the developed method, as little as 10 pmol of biologically active saccarides, such as blood type B antigen, pullulan, or the glucan of *Ganoderma lucidum* could be sensitively detected via MALDI-TOF MS. However, the labeling reaction was slightly time-consuming, and it was complete in 6 h.

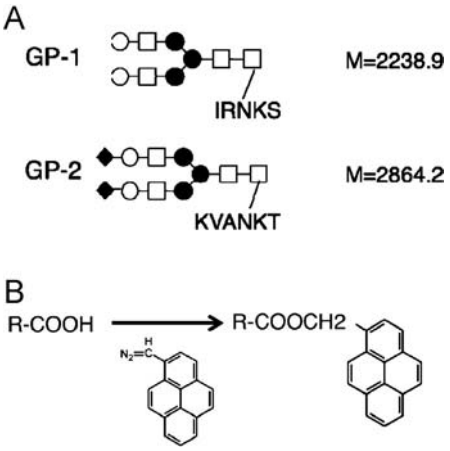


Fig. 7. (A) Structures of glycopeptides 1 and 2 (GP-1 and GP-2). (B) Reaction path of 1-pyrenyldiazomethane with carboxyl groups on peptides. Reprinted from [72], copyright 2010, with permission of American Chemical Society.

Lavanant and Loutelier-Bourhis [68] utilized procaine and procainamide to derivatize oligosaccharides with improved S/N ratio and eliminate the need to search for "sweet spots". Since the addition of procaine or procainamide did not impede the ionization process, thus, further purification step that could induce sample losses was eliminated. Furthermore, the labeling reaction is very rapid, and it could complete within 1 min at room temperature. Aminopyrazine [69] was further found that could be used as derivatization reagent and co-matrix to analyze oligosaccharides with improved ionization efficiency. For example, the detection sensitivity of maltoheptaose increased 10-fold via MALDI-TOF MS.

4.3. Glycopeptides

The main drawback of direct analysis of glycans or oligosaccharides is the information concerning glycosylation sites is lost. Thus, several tags have been reported to directly derivatize of glycopeptides, as summarized in Table 6.

Ullmer et al. [70] exploited to derivatize small peptides and glycopeptides with a commonly used fluorescent reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). AQC derivatization was allowed to proceed for 10 min at 55 °C with high reproducibility. It could increase the signal intensities by both MALDI-MS and ESI–MS analysis. For example, by combining with MALDI-TOF MS analysis, the LOD of the small glycopeptides from antithrombin could be lowered to about 500 fmol for direct analysis and 50 fmol after pre-enrichment via lection affinity chromatography.

Chamot-Rooke et al. [71] reported the derivatization of the N-terminus of peptides by (N-succinimidyloxycarbonylmethyl) tris(2,4,6-trimethoxyphenyl)phosphonium bromide. The reaction was proceeded in water bath for 30 min via sonication. Since a permanent positive charge was introduced, the sequence coverage for both O-glycosylated and O-phosphorylated peptides could be dramatically improved via electron capture dissociation (ECD) MS/MS. For model glycopeptides ALGST∗T∗PPA, ALGS∗T∗TPPA, GTTPSPVPT∗TSTTSAPG, and KGGGTTSTT∗SAPG, the sequence coverage could be increased to 75%, 75%, 75%, and 91.5% from 25%, 37.5%, 50%, and 75%; a nearly full sequence coverage was obtained for the tested peptides, considering that the Xxx-Pro is rarely cleaved in ECD MS/MS. Moreover, the exclusive presence of N-terminal fragments (c-type ions) could simplify the peptide sequence interpretation.

Amano and coauthors [72,73] proposed the on-plate pyrene derivatization method using 1-pyrenyldiazomethane that was suitable for sensitive MALDI MS analysis of glycopeptides even with the presence of non-glycopeptides, as shown in Fig. 7. Interestingly, the covalent-bonded tag could release from the glycopeptides via in-source decay. Thus, native form ions were observed from the MS spectra. Moreover, the derivatization could dramatically reduce the ionization efficiency of abundant peptides, revealing strong signals for low response glycopeptides. Glycopeptides from as little as 1 ng of prostate specific antigen were efficiently detected with the developed method.

5. Conclusion

In this review, the recent developed tags for efficient analysis of peptides by MS or MS/MS have been systematically summarized. Most of the tags were designed with strong gas-phase basicity, high hydrophobicity and/or a permanent positive charge. Ionization efficiency for some of the derivatized peptides could be increased up to 2000-fold, and LOD could decrease to low fmol. Especially, many tags were designed to be compatible with novel MS/MS fragmentation modes, such as ETD and ECD, which especially benefit analysis of peptides with PTMs. Moreover, several isotope introduced tags were synthesized for simultaneously quantitative and high efficient determination of peptides. However, up till now, most of these tags were only used for analysis of model peptides or tryptic digests of model proteins. We anticipate these tags will play important role in high-efficiency peptide analysis, and further qualitative and quantitative proteome research in the near future.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21205027), National Basic Research Program of China (2012CB910604) and Natural Science Foundation of Hebei Province (B2012201095, B2012201052).

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