Mol Divers (2012) 16:613–618
DOI 10.1007/s11030-012-9377-y

SHORT COMMUNICATION

Sensitive electrospray mass spectrometry analysis of one-bead-one-compound peptide libraries labeled by quaternary ammonium salts

Remigiusz Bąchor · Marzena Cydzik · Magdalena Rudowska · Alicja Kluczyk · Piotr Stefanowicz · Zbigniew Szewczuk

Received: 17 April 2012 / Accepted: 28 May 2012 / Published online: 28 June 2012

Abstract A rapid and straightforward method for high-throughput analysis of single resin beads from one-bead-one-compound combinatorial libraries with high resolution electrospray ionization tandem mass spectrometry (HR ESI-MS/MS) is presented. The application of an efficient method of peptide derivatization by quaternary ammonium salts (QAS) formation increases ionization efficiency and reduces the detection limit, allowing analysis of trace amounts of compounds by ESI-MS. Peptides, synthesized on solid support, contain a new cleavable linker composed of a Peg spacer (9-aza-3,6,12,15-tetraoxa-10-on-heptadecanoic acid), lysine with ε-amino group marked by the *N*,*N*,*N*-triethylglycine salt, and methionine, which makes possible the selective cleavage by cyanogen bromide. Even a small portion of peptides derivatized by QAS cleaved from a single resin bead is sufficient for sequencing by HR ESI-MS/MS experiments. The developed strategy was applied to a small training library of α chymotrypsin substrates. The obtained results confirm the applicability of the proposed method in combinatorial chemistry.

Keywords Derivatization of peptides · ESI-MS/MS · Fixed charge tag · Linker design · OBOC · Quaternary ammonium salts

Electronic supplementary material The online version of this article (doi:10.1007/s11030-012-9377-y) contains supplementary material, which is available to authorized users.

R. Bąchor · M. Cydzik · M. Rudowska · A. Kluczyk · P. Stefanowicz · Z. Szewczuk (✉)
Faculty of Chemistry, University of Wrocław, ul. F. Joliot-Curie 14, 50-383 Wrocław, Poland
e-mail: zbigniew.szewczuk@chem.uni.wroc.pl

Introduction

Rapid and efficient methods of combinatorial peptide library synthesis allow for obtaining a wide range of compounds in short time. Solid phase synthesis using the "split-and-mix" technique has become a method of choice in preparation of One-Bead-One-Compound (OBOC) libraries [1,2]. The advantage of the OBOC method is that thousands (or even millions) of peptidic compounds can be rapidly synthesized and screened for biological activity. Once a peptide library has been screened against the interesting target, the chemical identification of the peptides on the hit beads is required. Chemical structure of the peptide attached to the selected bead can be identified and analyzed by mass spectrometry (MS) methods in a single experiment without time consuming deconvolution, required in other combinatorial library methods [3].

Several analytical techniques such as IR, NMR, MS or Edman degradation have been widely used in OBOC combinatorial chemistry. The direct analysis of resin-bound peptides by Edman degradation is expensive and time consuming, making it impractical for the routine sequencing of large numbers of peptides (>100) [4]. Furthermore, this technique is not generally applicable as it requires free N-terminal amino group and is limited by the peptide chain length [5]. However, the fundamental problem of OBOC peptide library analysis is the small amount of compound obtained from a single resin bead and insufficient ionization efficiency of some peptides for standard ESI-MS analysis. This may result in the incomplete sequence coverage during the analysis, which makes peptide mass fingerprinting investigations problematical. Therefore, an enhanced detectability is an important goal of mass spectrometric analysis. One of the main approaches for increasing the ionization efficiency is application of fixed charge tags [6]. Several peptide charge

derivatization strategies such as quaternary ammonium [7], sulphonium [8–10], and phopshonium [11] salts formation have been developed. These modifications were performed in solution and, therefore, are not appropriate for analysis of OBOC libraries. Previously, Carrasco et al. [12] described application of multidetachable linkers for direct monitoring of organic compounds on solid support. The construct consists of a photocleavable linker, and a sequence containing betaine-ionization tags to enhance ionization of poorly- or non-ionizable molecules by MALDI mass spectrometry. However, it was shown that the introduction of trimethylammonium salt increase the ionization efficiency in a small range [13]. Recently, we developed an efficient and straightforward method for formation of various quaternary ammonium salts (QAS) on solid support [14]. The modification increases ionization efficiency of peptides in electrospray mass spectrometry (ESI-MS) and lowers the detection limit, whereas a fixed charge tag in MS/MS experiment is responsible for specific fragmentation patterns, which makes it very useful in the analysis of OBOC peptide libraries. Our previous study evidenced that in presence of QAS as a fixed charge tag peptide may fragment via both charge remote and charge directed mechanism [15].

This article presents synthesis and application of a new linker containing QAS group, for OBOC peptide libraries, and HR ESI-MS/MS analysis of a trace amount (less than 10 %) of peptides obtained from a single resin bead. The introduction of stable positive charge enables peptide identification at low femtomolar level [14], which may be particularly useful in OBOC peptide libraries analysis, since the remaining portion of material isolated from the single bead could be used for other experiments.

Results

For the preparation of the OBOC peptide libraries, a resin modified by a new linker with -Gly-Peg-Lys(Mtt)-Met-Peg- sequence was used. The two flexible and hydrophilic Peg residues (18-atom each) were introduced to separate peptidic substrates both from the resin core and fixed charge tag located at the ε-amino group of lysine residue. The enzymatic digestion of peptides immobilized on the resin bead is usually limited by the availability of substrate and the ability of enzyme to penetrate the resin. This may cause erroneous results during substrate profiling. Therefore, the appropriate spacer, which increases the peptide availability for enzymes, is needed. The spacer should possess hydrophilic properties, because enzymatic digestion usually occurs in water. Moreover, its length should also sufficiently increase the distance between peptide and the resin. These two requirements can be met by using linkers containing polyethylene glycol derivatives, which are commonly used in OBOC library of protease substrates [16].

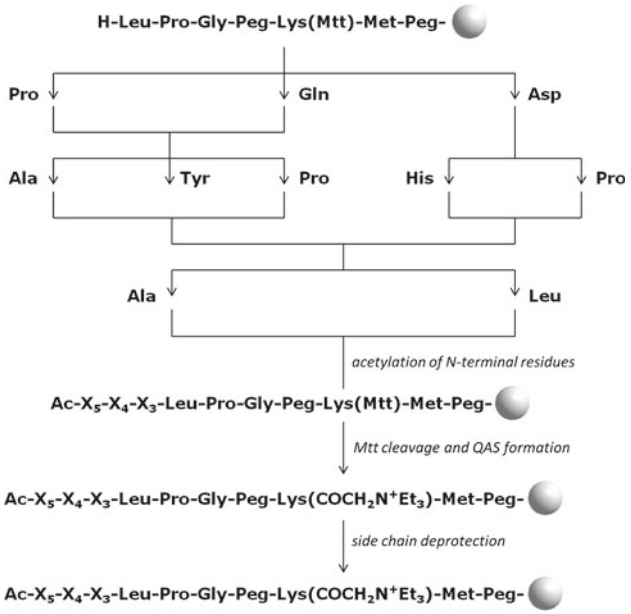


Fig. 1 Scheme of synthesis of model peptide library of the α chymotrypsin substrates with a new QAS-tagged linker susceptible to BrCN cleavage. *Gray ball* indicates a single bead of TentaGel resin

The methionine residue allows for selective and efficient peptide cleavage in the reaction with cyanogen bromide. The limitation of this approach is that methionine residue is excluded from the peptide library. Although several chemical and photochemical cleavage strategies have been developed [17], the introduction of a BrCN-cleavable methionine-linker group at the C-terminus of the OBOC peptide library is very common [18].

Combinatorial peptide library preparation

As a proof of concept a small 16-member training library of α chymotrypsin substrates was designed and synthesized on TentaGel resin. A pentapeptide fragment with the Leu-Tyr-Gln-Leu-Glu sequence, corresponding to the insulin A chain (13-17), which was identified as a chymotrypsin substrate [19] responsible for amyloids plaque formation [20], was used as a model peptide. All peptides contain proline residue in X_1 position and leucine in X_2 position. Peptide library was modified in position X_3 (Gln, Pro, Asp), X_4 (Ala, Tyr, Pro, His) and X_5 (Leu, Ala) (see Fig. 1). This produced a 16-peptide library in five synthetic steps. Most of the library components were α chymotrypsin substrates. Peptide sequences (Table 1) were selected in order to conduct substrate analysis for α chymotrypsin and compared with those described by Keil [21]. Because the substrate specificity of used enzyme is well known, the main goal of designed library was to verify the compatibility of our derivatization strategy in combinatorial chemistry and to determine the peptide sequences obtained from both, the positive and negative hits.

Combinatorial peptide library was synthesized according to a standard Fmoc strategy and "split-and-mix" method (Fig. 1). The N-terminal amino groups were capped by the reaction with acetic anhydride.

QAS in a form of *N*,*N*,*N*-triethylglycine were efficiently prepared on solid support after the peptide library synthesis and the Mtt-group removal from the ε-amino group of lysine residue by 1 % TFA in DCM. Iodoacetic acid was coupled to the amino group in the presence of DIC. The nucleophilic substitution of iodine atom by triethylamine led to triethylammonium salt. The reaction was completed within 24 h. The strategy of *N*, *N*, *N*-triethylglycine introduction to the lysine side chain was similar to described by us previously [14]. The remaining side chain protecting groups were cleaved by the mixture of TFA/TIS/H_2O (95:2.5:2.5; v:v:v) for 2 h. Under these conditions, the peptides chains were not removed from the resin.

Application of QAS containing linker to OBOC library

The prepared combinatorial peptide library was subjected to enzymatic digestion by α chymotrypsin. This serine protease was selected, since it is one of the most frequently used enzymes in screening studies [22]. Proteolysis of peptide bonds results in formation of free amino groups, which are known to yield a purple color (Ruhemann's purple) in the presence of ninhydrin. This allows for positive beads identification by observation of color change, and enables selection of beads containing chymotrypsin substrates. The analysis of peptides obtained from single resin beads was performed by HR ESI-MS/MS.

Ninhydrin is used to monitor deprotection of primary amino groups in solid phase peptide synthesis (Kaiser test). When even a small portion (less than 1 %) [23] of amino groups is deprotected, a ninhydrin test yields purple product. Colorless or yellow beads suggest complete acylation of all primary amino groups. Acetylation of N-terminal amino acid residues produces beads not reacting with ninhydrin. Proteases cause hydrolysis of specific peptide bonds and, as a result, a free amino group appears in the peptide stub left attached to the resin bead. Even if the enzymatic digestion does occur incompletely, there should be enough of released primary amino groups to be detected by Kaiser test. The low yield proteolysis is actually an advantage, since it does not reduce significantly the amount of the intact peptide on the bead available for analysis. The limitation of the proposed method is that lysine and arginine cannot be included in library sequences, since their side chains give color products with ninhydrin. Recently, Kofoed and Reymond [24] proposed an alternative selective labeling of the free N-terminus by reductive alkylation with 4-carboxybenzaldehyde coupled to Disperse Red 1 in the presence of $NaBH_3CN$. The reagent can selectively stain-free amino groups liberated by proteolysis of peptides on TentaGel beads, even in presence of guanidine group of arginine residue. However, in this method lysine residue must also be excluded from the resin, since the reagent may stain the ε-amino group.

After the Kaiser test 45 purple resin beads (positive hits) and 25 yellow resin beads (negative hits) were selected for HR ESI-MS/MS analysis. Each bead was treated independently by cyanogen bromide in formic acid for 18 h, releasing peptides combined with the linker bearing QAS group.

It was expected that peptides containing tyrosine in X_4 position (which corresponds to P1 position according to Schechter and Berger nomenclature [25]) should be digested easily, while the presence of aspartic acid in X_3 position (P1′ in Schechter and Berger nomenclature) should inhibit the proteolysis. The proteolysis should also take place in the Leu-X sequence (where X is any amino acid). However, as the leucine is less preferred P1 residue, the influence of P1′ and P2′ is more pronounced, which may explain the result for peptide 8 [21]. In ninhydrin test, secondary amines as proline do not provide purple hits. Therefore, only peptide 4, which contains no tyrosine was identified as positive hit, because of the Leu–Ala sequence. Results of the performed ESI-MS, experiments confirmed by the fragmentation analysis (Spectra 1–32, Supplementary material), were in full accordance with these assumptions. The MS analysis of purple resin beads showed the presence of peptides 4, 11, 13,15 and 16, however the color in case of peptide 4 was less intense. Unchanged yellow resin beads (negative hits), yielded peptides 1–3, 5–7, 9,10, 12 and 14 as identified by MS analysis. We also observed grayish blue resin beads which contained peptide 8 (Table 1). Our results also indicate that presence of QAS group in the linker of the designed substrate library does not affect the enzymatic digestion.

The obtained mass spectra (Supplementary materials) show signals corresponding to the undigested peptides, which may suggest that only small amount of the available peptides was digested on the resin, although there is enough of released primary amino groups to be monitored by ninhydrin test. This may imply that the proteolysis occurs only on the surface of the resin bead, due to the limited penetration of the enzyme. However, small cyanogen bromide molecules can penetrate deeper into the resin bead, effectively cleaving peptide from the support. The low yield of the direct proteolysis of combinatorial peptide libraries on a solid support is an advantage of the method, since it does not reduce significantly the amount of the peptide available for analysis. Even less than 10 % of the peptide material obtained from one bead is enough to perform unambiguous test, because the QAS labeling method allows for the peptide analysis even at low femtomolar level by standard ESI-MS analysis [14].

We recently found that application of a nano-ESI-MS instrument further increases sensitivity of the analysis of the QAS peptides to low attomolar range (unpublished results).

Table 1 Kaiser test results of components of analyzed peptide library

Nr	Kaiser test for peptide bead	Peptide released from the bead
1	Yellow	Ac-Ala-Ala-Pro-Leu-Pro-LINKER[a]
2	Yellow	Ac-Ala-Pro-Pro-Leu-Pro-LINKER
3	Yellow	Ac-Ala-Ala-Gln-Leu-Pro-LINKER
4	Purple	Ac-Leu-Ala-Pro-Leu-Pro-LINKER
5	Yellow	Ac-Ala-Pro-Asp-Leu-Pro-LINKER
6	Yellow	Ac-Ala-Pro-Gln-Leu-Pro-LINKER
7	Yellow	Ac-Leu-Pro-Pro-Leu-Pro-LINKER
8	Grayish blue	Ac-Leu-Ala-Gln-Leu-Pro-LINKER
9	Yellow	Ac-Ala-His-Asp-Leu-Pro-LINKER
10	Yellow	Ac-Leu-Pro-Asp-Leu-Pro-LINKER
11	Purple	Ac-Ala-Tyr-Pro-Leu-Pro-LINKER
12	Yellow	Ac-Leu-Pro-Gln-Leu-Pro-LINKER
13	Purple	Ac-Ala-Tyr-Gln-Leu-Pro-LINKER
14	Yellow	Ac-Leu-His-Asp-Leu-Pro-LINKER
15	Purple	Ac-Leu-Tyr-Pro-Leu-Pro-LINKER
16	Purple	Ac-Leu-Tyr-Gln-Leu-Pro-LINKER

Sequences of peptides identified according to MS/MS experiments (Supplementary materials).
HSL homoserine lactone
[a] LINKER -Gly-Peg-Lys ($COCH_2N^+Et_3$)-HSL

We have observed a phenomenon that signals representing the doubly charged ions $[M+H]^{2+}$ corresponding to the protonated peptides bearing QAS-tag have higher intensity than those with single charge (Spectrum 1) (Spectra 1–16, Supplementary data). Therefore, the doubly charged signals were chosen for fragmentation (Spectrum 2) (Spectra 17–32, Supplementary data).

The ESI-MS/MS Spectrum 2 shows a clear series of y-type ions of analyzed compound. Only low-abundance peaks, corresponding to the b-type ions were observed. Previously, we have found that introduction of QAS residue attached to peptide N-terminus produces more complicated ESI-MS/MS spectra [14]. Fragmentation related to the presence of fixed charge tag at the C-terminus facilitates the MS/MS spectrum interpretation, since mainly one type of daughter ions are observed. No peaks corresponding to the QAS group fragmentation or elimination were observed. Fragmentation spectra recorded for other library members are presented in Supplementary data.

The results of our work indicate that the application of QAS-tag for OBOC combinatorial libraries, and HR ESI-MS/MS method increases the sensitivity of detection thus enabling identification of a trace amount (less than 10 %) of peptides obtained from a single resin bead. The presence of almost exclusively one fragmentation ion series in the mass spectrum facilitates the interpretation and sequence analysis of released peptides.

In another project, QAS peptides containing β-alanine and γ-aminobutyric acid as well as longer peptides up to 14 residues were synthesized and successfully analyzed using mass spectrometry thus confirming the general application of our method (manuscript in preparation). This rapid and straightforward method for high-throughput analysis of single resin beads was used for a training library of α chymotrypsin substrates and confirmed that the presented methodology may be particularly useful in OBOC peptide library analysis.

Experimental section

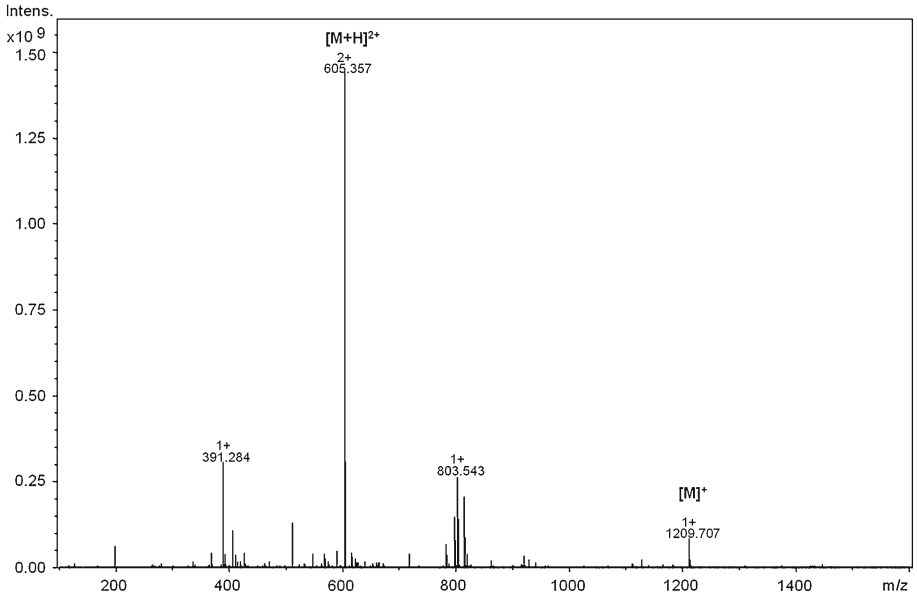
Synthesis

Synthesis of combinatorial libraries was performed manually by "split-and-mix" method (Fig. 1) on solid support according to a standard Fmoc synthesis procedure [26]

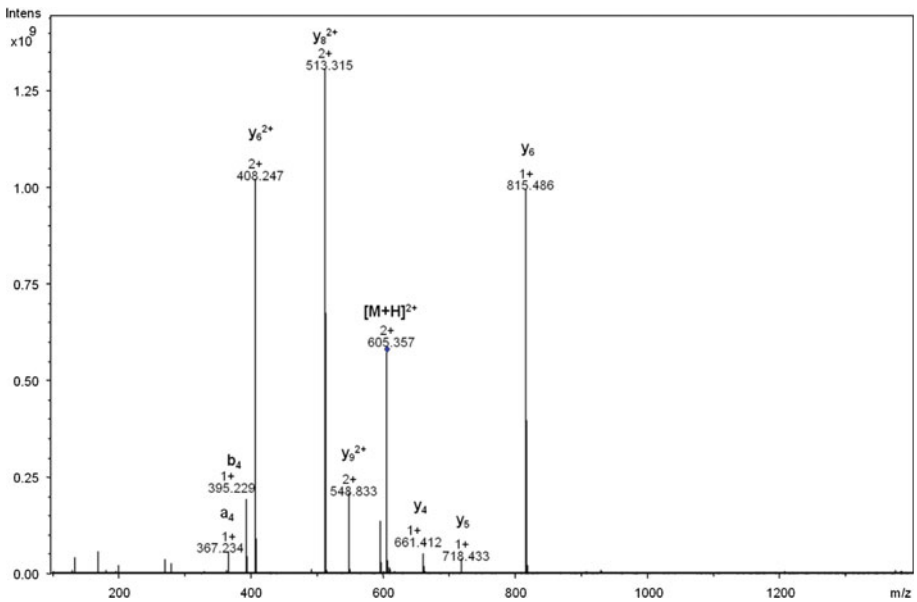
The N-terminus was acetylated (Ac_2O/DMF/DIEA; 1:7.5:1.5; v:v:v), to allow hit identification after enzymatic digestion by Kaiser test [23].

QAS formation

After peptide synthesis (on the TentaGel HL-NH_2 resin) the ε-amino group of lysine residue was derivatized on solid support to form QAS. Mtt protecting group was cleaved by using 1 % solution of TFA in DCM (3×2 min, 3×10 min, 3 × 2 min). Then the peptidyl resin was washed with DCM (3 × 1 min), DCM/DMF (1:1; v:v, 1 min), 5 % DIEA in DMF (3 × 1 min) and DMF (7 × 1 min). The mixture of iodoacetic acid (26 mg, 140 μmol) and DIC (17.7 mg, 140 μmol), dissolved in DMF (0.5 mL), was added to the peptidyl resin (50 mg, 28 μmol) and the reaction was allowed to proceed for 3 h. Then triethylamine (75.6 μL, 560 μmol), disolved in



Spectrum 1 HR ESI-MS spectrum of compound **1**. The ESI-MS spectrum of analyzed compound shows, that signal intensity corresponding to the peptide with additional proton $[M+H]^{2+}$ is higher than that of singly charged



Spectrum 2 HR ESI-MS/MS spectrum of compound **1**. Parent ion was 605.357 $[M+H]^{2+}$

DMF (0.5 mL), was added to the reaction vessel and mixed for 24 h. Side chain protecting groups were cleaved with the mixture of TFA/TIS/H_2O (95:2.5:2.5; v:v:v) for 2 h and the peptidyl resin was washed with 5 % solution of DIEA/DMF (3 × 1 min), DMF (6 × 1 min), DMF/DCM (1:1; v:v, 1 min), DCM (6 × 1 min), DCM/MeOH (1:1; v:v, 1 min), MeOH (6 × 1 min) and dried in vacuo.

Enzymatic digestion

The peptidyl resin (5 mg) was washed with water (3 × 1 min) and 0.01 M ammonium bicarbonate in water (3 × 1 min). Enzymatic digestion was performed in 0.01 M ammonium bicarbonate buffer for 3 h in 37 °C. The peptidyl resin to enzyme ratio was 1,000:1. The reaction was terminated by the addition of formic acid (3 μL). After the supernatant separation, the resin was washed with water (3 × 1 min), acetonitrile (3 × 1 min) and methanol (3 × 1 min). Then two drops of Kaiser components (A: 80 g phenol in 20 mL ethanol, B: 2 mL 0.001 M KCN in 98 mL pyridine, and C: 5 g of ninhydrin in 100 mL ethanol) [23] were added and incubated for 3 min at 100°C. The resin was washed with methanol (3 × 1 min) and DMF (3 × 1 min). Positive (purple) and negative (yellow) hits were separated manually following by washing with water (3 × 1 min), acetonitrile (3 × 1 min), methanol (3 × 1 min), H_2O (3 × 1 min) and 70 % HCOOH (3 × 1 min).

Then each selected bead was treated individually with 0.25 M BrCN in 70 % formic acid (20 μL) for 18 h, according to the method described by Franz et al. [27] 10 % of the obtained peptide-homoserine lacton was dissolved in the mixture (70 μL) of water, acetonitrile, and formic acid (50:50:0.1; v:v:v) and analyzed directly by HR ESI-MS.

Mass spectrometry

All MS experiments were performed on an FT-ICR (Fourier Transform Ion Cyclotron Resonance) MS Apex-Qe Ultra 7T instrument (Bruker Daltonics, Germany) equipped with standard ESI source. The instrument parameters were as follows: scan range: 100–1,600 m/z; drying gas: nitrogen; flow rate: 1.5 l/min; analyte solutions (70 μl) were introduced at a flow rate of 3 μl/min; temperature: 200°C; potential between the spray needle and the orifice: 4.2 kV.

Acknowledgments This work was supported by a Grant No. N N204 180040 from the Ministry of Science and Higher Education of Poland.

References

1. Lam KS, Salmon SE, Hersh EM, Hruby VJ, Kazmierski WM, Knapp RJ (1991) A new type of synthetic peptide library for identifying ligand-binding activity. Nature 354:82–84. doi:10.1038/354082a0
2. Furka A, Sebestyen F, Asgedom M, Dibo G (1991) General method for rapid synthesis of multicomponent peptide mixtures. Int J Pept Protein Res 37:487–493. doi:10.1111/j.1399-3011.1991.tb00765.x
3. Aina OH, Liu R, Sutcliffe JL, Marik J, Pan CX, Lam KS (2007) From combinatorial chemistry to cancer-targeting peptides. Mol Pharm 4:631–651. doi:10.1021/mp700073y
4. Wang P, Arabaci G, Pei D (2001) Rapid sequencing of library-derived peptides by partial edman degradation and mass spectrometry. J Comb Chem 3:251–254. doi:10.1021/cc0001021
5. Redman JE, Wilcoxen KM, Ghadiri MR (2003) Automated mass spectrometric sequence determination of cyclic peptide library members. J Comb Chem 5:533–540. doi:10.1021/cc0200639
6. Kidwell DA, Ross MM, Colton RJ (1984) Sequencing of peptides by secondary ion mass spectrometry. J Am Chem Soc 106:2219–2220. doi:10.1021/ja00319a057
7. Mirzaei H, Regnier F (2006) Enhancing electrospray ionization efficiency of peptides by derivatization. Anal Chem 78:4175–4183. doi:10.1021/ac0602266
8. Samgina TY, Kovalev SV, Gorshkov VA, Artemenko KA, Poljakov NB, Lebedev AT (2010) N-terminal tagging strategy for De Novo sequencing of short peptides by ESI-MS/MS and MALDI-MS/MS. J Am Soc Mass Spectrom 21:104–111. doi:10.1016/j.jasms.2009.09.008
9. Stefanowicz P (2004) Electrospray mass spectrometry and tandem mass spectrometry of the natural mixture of cyclic peptides from linseed. Eur J Mass Spectrom 10:665–671. doi:10.1255/ejms.657
10. Reida GE, Roberts KD, Simpson RJ, O'Hair RAJ (2005) Selective identification and quantitative analysis of methionine containing peptides by charge derivatization and tandem mass spectrometry. J Am Soc Mass Spectrom 16:1131–1150. doi:10.1016/j.jasms.2005.03.015
11. Zaia J, Biemann K (1995) Comparison of charged derivatives for high energy collision-induced dissociation tandem mass spectrometry. J Am Soc Mass Spectrom 6:429–436. doi:10.1016/1044-0305(95)00018-9
12. Carrasco MR, Fitzgerald MC, Oda Y, Kent SBH (1997) Direct monitoring of organic reactions on polymeric supports. Tetrahedron Lett 38:6331–6334. doi:10.1016/S0040-4039(97)01456-1
13. Vath JE, Biemann K (1990) Microderivatization of peptides by placing a fixe positive charge at the n-terminus to modify high energy collision fragmentation. Int J Mass Spectrom Ion Process 100:287–299. doi:10.1016/0168-1176(90)85079-H
14. Cydzik M, Rudowska M, Stefanowicz P, Szewczuk Z (2011) Derivatization of peptides as quaternary ammonium salts for sensitive detection by ESI-MS. J Pept Sci 17:445–453. doi:10.1002/psc.1342
15. Cydzik M, Rudowska M, Stefanowicz P, Szewczuk Z (2011) The competition of charge remote and charge directed fragmentation mechanisms in quaternary ammonium salt derivatized peptides—an isotopic exchange study. J Am Soc Mass Spectrom 22:2103–2107. doi:10.1007/s13361-011-0245-2
16. Grøtli M, Gotfredsen ChH, Rademann J, Buchardt J, Clark AJ, Duus JØ, Meldal M (2000) Physical properties of poly(ethylene glycol) (PEG)-based resins for combinatorial solid phase organic chemistry: a comparison of PEG-cross-linked and PEG-grafted resins. J Comb Chem 2:108–119. doi:10.1021/cc990048c
17. Peukert S, Giese B (1998) The pivaloylglycol anchor group: a new platform for a photolabile linker in solid-phase synthesis. J Org Chem 63:9045–9051. doi:10.1021/jo9816055
18. Lee SS, Lim J, Cha J, Tan S, Heath JR (2008) Rapid microwave-assisted CNBr cleavage of bead-bound peptides. J Comb Chem 10:807–809. doi:10.1021/cc800113d
19. Schilling RJ, Mitra AK (1991) Degradation of insulin by trypsin and alpha-chymotrypsin. Pharm Res 8:721–727. doi:10.1023/A:1015893832222
20. Ivanova MI, Thompson MJ, Eisenberg DA (2006) Systematic screen of beta(2)-microglobulin and insulin for amyloid-like segments. Proc Natl Acad Sci USA 103:4079–4082. doi:10.1073/pnas.0511298103
21. Keil B (1992) Specificity of proteolysis. Springer, Berlin
22. Hileman OE, Brook M (2005) The use of poly(ethylene oxide) for the efficient stabilization of entrapped alpha-chymotrypsin in silicone elastomers: a chemometric study. Biomaterials 26:6973–6983. doi:10.1016/j.biomaterials.2005.05.016
23. Kaiser E, Colescott RL, Bossinger CD, Cook PI (1970) Color test for detection of free terminal amino groups in the solid-phase synthesis of peptides. Anal Biochem 34:595–598. doi:10.1016/0003-2697(70)90146-6
24. Kofoed J, Reymond JL (2007) Identification of protease substrates by combinatorial profiling on TentaGel beads. Chem Commun 43:4453–4455. doi:10.1039/b713595e
25. Schechter I, Berger A (1967) On the size of the active site in proteases. I. Papain. Biochem Biophys Res Commun 27:157–162. doi:10.1016/S0006-291X(67)80055-X
26. Chan WC, White PJ (2000) Fmoc solid phase peptide synthesis: a practical approach. Oxford University Press, New York
27. Franz AH, Liu R, Song A, Lam KS, Lebrilla CB (2003) High-throughput one-bead-one compound approach to peptide-encoded combinatorial libraries: MALDI-MS analysis of single TentaGel beads. J Comb Chem 5:125–137. doi:10.1021/cc020083a