Chinese Chemical Letters Vol. 15, No. 6, pp 652– 654, 2004
http://www.imm.ac.cn/journal/ccl.html

Construction and Analysis of N-phosphoryl Peptide Libraries

Shu Xia CAO[1], Jian Chen ZHANG[1], Ming Yu NIU[1],
Kui LU[2], Xin Cheng LIAO[1], Yu Fen ZHAO[1,3]*

[1]The Key Laboratory of Chemical Biology, Department of Chemistry, Zhengzhou University, Zhengzhou 450052
[2] Department of Chemistry and Chemical Engineering, Zhengzhou Institute of Technology, Zhengzhou 450052
[3] The Key Laboratory for Bioorganic Phosphorus Chemistry and Chemical Biology, Ministry of Education, Department of Chemistry School of Life Science and Engineering, Tsinghua University, Beijing 100084

Abstract: N-Phosphoryl peptide libraries were constructed by transformation from homo-oligopeptide libraries, which was synthesized by self-assembly of amino acids with the assistance of phosphorus oxychloride. Electrospray ionization mass spectrometry (ESI-MS) was used to monitor the reaction.

Keywords: N-Phosphoryl peptide library, homo-oligopeptide library, electrospray ionization mass spectrometry (ESI-MS).

Chemical diversities on solution phase combinatorial chemistry (SPCC) have attracted tremendous attention because of their potential application in rapid drug discovery[1]. N-Phosphoryl peptide represents a class of unique bioactive material. Therefore, it is meaningful to introduce these functionalities into peptide libraries to enhance the possibility of discovering new drug and to enhance diversity of peptide libraries. We have reported that α-amino acids could be assembled into homo-peptide libraries with the assistance of phosphorus oxychloride[2]. To expand the diversity of compounds which can be synthesized combinatorial, the principles of switchover design were used to change a library by functional group transformation[3]. In this paper we firstly describe an establishment of N-diisopropyloxyphosphoryl peptide libraries by transformation from homo-peptide libraries. This post-modification strategy has the potential to be extended to other types of reaction for altering peptide and may allow greater chemical diversity. ESI-MS and MS/MS are powerful tools for monitoring the synthetic reaction[4] and the products from combinatorial chemistry[5]. Compounds synthesized in this method were characterized by ESI-MS/MS.

The reaction is shown in **Scheme 1**. Analysis of the reaction solution by ESI-MS and MS^n showed that after L-amino acids reacted with $POCl_3$ for only one hour and quenching with H_2O, a series of mass peaks corresponding to oligomeric peptides of

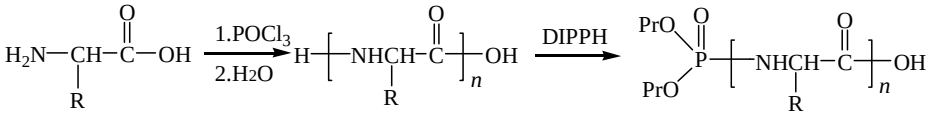
* E-mail: zicb@zzu.edu.cn

amino acids were already observed. Not only in positive spectra but also in negative spectra, it was observed that the length of the peptide slowly increased as reaction time prolonged.

Above homo-oligopeptide crude products were transformed to N-diisopropyloxy-phosphoryl conjugated peptide libraries by the post-modification strategy. Diisopropyl phosphite (DIPPH) was used as a phosphoryl agent and the gel column (Sephadex LH 20) was used as a purification tool[6]. It had been reported that the response sensitivity could be improved greatly by introducing the N-dialkyloxyphosphoryl group into amino acids in ESI-MS[7], so the negative mode was used to detect N-phosphoryl peptide library. As shown in **Figure 1**, which was the analytic results of L-alanine (L-Ala), the peak at *m/z* 323, 394, 465, 536, 607, 678, 749, 820, 891 *etc.* were identified as N-phosphoryl deprotonated oligopeptide of $(Ala)_n$-OH (n=2, 3, 4, 5, 6, 7, 8, 9, 10 respectively).

To identify the product structures of N-phosphoryl peptide library, the MS/MS spectra of $[M-H]^-$ ions were investigated by collision-induced dissociation (CID) tandem mass spectrometry. The main fragment ions of $[M-H]^-$ ion were $[M-H-C_3H_6-H_2O]^-$ and $[M-H-2C_3H_6-H_2O]^-$. For example, the $[M-H]^-$ ion of DIPP-Ala-Ala at *m/z* 323 produced the fragment ion at *m/z* 263 corresponding to the $[M-H-C_3H_6-H_2O]^-$. The ion at *m/z* 263 then lost the second propylene to form the ion at *m/z* 221. In the same way, the $[M-H]^-$ ion of DIPP-Ala-Ala-Ala at *m/z* 394 produced the fragment ion at *m/z* 334 and *m/z* 292, respectively.

Scheme 1 Synthetic procedure for the N-phosphoryl peptide libraries

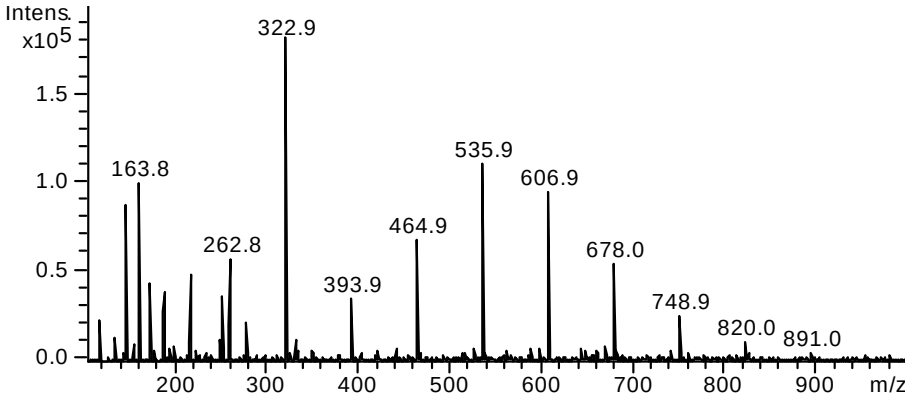


R= Gly, L-Ala, L-Leu, L-Ile, L-Val, L-Phe

n = 2-13

n = 2-13

Figure 1 Negative ESI-MS spectra of N-phosphoryl peptide library from L-ala homo-oligopeptide



In conclusion, the post-modification of peptide on the solution phase has been developed to introduce phosphoryl group. This is a convenient method for generating small libraries of biologically active N-phosphoryl peptide and the method for separation was initiatively explored. Construction of N-phosphoryl conjugated peptide libraries is firstly described and this approach has the potential to enhance chemical diversity.

Acknowledgment

We are grateful to the National Natural Science Foundation of China (NO. 20272055) for support of this research.

References and Notes

1. H. An, P. D. Cook, *Tetrahedran Lett.*, **1996**, *37*, 7233.
2. N. Zhou, K. Lu, Y. Liu, *et.al.*, *Rapid Commun. Mass Spectrom.*, **2002,** *16*, 919.
3. Y. Pei, W. H. Moos, *Tetrahedran Lett.*, **1994**, *35*, 5825.
4. C. J. Zhu, X. L. Yang, S. X. Cao, *et al*., *Rapid Commun. Mass Spectrom.*, **2003**, *17*, 825.
5. Y. G. Shin, R. B. van Breemen, *Biopharmaceutics & Drug Disposion*, **2001**, *22*, 353.
6. Typical reaction produre: Homo-oligopeptide crude products were dissolved in 10 mL deionized water, 10 mL triethylamine, 5 mL ethanol and cooled in ice-salt bath to 0℃. 5 mmol DIPPH, 10 mL tetrachloromethane were added into the mixture and stirred for 3 hours. The mixture was extracted with ether(2×10 mL) and the water layer was adjusted to pH=3~4 with 1 mol/L hydrochloric acid in ice-salt bath，then fully extracted with ethyl acetate（5×10 mL）. Oily liquids were acquired after removal of solvents. The gel column (Sephadex LH 20) loaded with crude library products was eluted with methanol to remove the impurities with less molecular weight. All fractions containing library compounds were collected to get more pure library products.
7. Y. Jiang, H. Fu, L. Xu, Q. Lu, J. Z. Wang, Y. F. Zhao, *Rapid Commun. Mass Spectrom.*, **2000**, *14*, 1491.

Received 17 June, 2003