# LC-MS analysis of the formed peptides from *N*-(*O*,*O*-diisopropyl)phosphoryl aspartic acid

HU, Jian-Jun(胡建军) JU, Yong(巨勇) ZHAO, Yu-Fen*(赵玉芬)
*Bioorganic Phosphorus Chemistry Laboratory, Department of Chemistry, School of Life Science and Engineering, Tsinghua University, Beijing 100084, China*

**LC-ESI-MS method was used to analyze the formed di- and tri- peptide in the reaction system of *N*-(*O*, *O*-diisopropyl) phosphoryl aspartic acid and adenosine. Cluster ions of the peptides were given in the ESI-MS. The structures of these small peptides were confirmed by LC-MS-MS analysis. Compared with the traditional HPLC-UV detection, this method showed good sensitivity and selectivity for peptide in the presence of compounds with strong UV absorption, such as nucleoside and nucleotide.**

**Keywords** LC-ESI-MS, peptide, *N*-phosphoryl amino acid, tandem mass spectrometry, cluster ion

## Introduction

*N*-(*O*, *O*-Dialkyl) phosphoryl amino acids (DAP-AA) have been found to possess many chemical and biochemical activities. For example, they could self-activate to form peptides, react with nucleoside to give nucleotide and oligonucleotide, ester-exchange and be esterfied with alcohol and some hydroxyl amino acids such as DAP-serine could proceed the nitrogen-to-oxygen phosphoryl migration reaction.[1-8] Since all of these reactions occurred without the participation of any enzyme, any energy carrier (such as ATP) or any condensing reagent, *N*-(*O*, *O*-dialkyl)phosphoryl amino acid (DAP-AA) was proposed as a model compound for the common origin for the prebiotic protein and nucleic acid.[9,10] In the reaction of DAP-AA with nucleoside, the peptide formation reaction should also occur besides the nucleotide formation process. Usually, for the detection of the nucleotide and nucleoside, HPLC-UV is a very powerful method. However, in these reaction systems the presence of nucleotide and nucleoside with strong UV absorption at 254 and 210 nm will suppress the amino acid and peptide, which have much weaker UV absorption. Hence, it is necessary to look for some other methods to detect the peptide in these reaction systems.

Mass spectrometry is a very important and powerful technique to obtain the molecular weight and structure of the products. Recently, LC-MS technology has been developed extensively and was applied to biochemical and biotechnological areas in the last decade. Especially, the LC-MS-MS spectrometry with electrospray ionization (ESI) interface was very useful for the characterization of peptide and proteins.[11-18] In this paper, LC-MS-MS was employed to study the peptide formation reaction of *N*-(*O*, *O*-diisopropyl) phosphoryl aspartic acid in the presence of adenosine in aqueous medium.

## Experimental

### *Chemicals*

Aspartic acid and adenosine were purchased from Sigma. Acetonitrile was of HPLC grade. Water for the mobile phase was deionized. Other chemicals and reagents were of AR grade.

Received April 3, 2000; accepted June 7, 2000.
Project supported by the National Natural Science Foundation of China (No. 39870415 and 29672022), National Science and Technology Committee of China, Education Ministry of China and Tsinghua University.

*Methods*

$N$-( $O$, $O$-Diisopropyl)phosphoryl aspartic acid (0.5 mmol), which was prepared according to literature method,[19,20] was dissolved in 0.5 mL of water-pyridine ($V/V = 3:2$) and 5 mg of adenosine was added. The mixture was incubated at 40°C for 4 days and then was analyzed with LC-ESI-MS.

*Apparatus*

LC-ESI-MS was performed on the triple quadrupole PE SCIEX API2000 LC/MS/MS mass spectrometer system from Perkin-Elmer Sciex Instruments. Mass spectra were recorded in positive ion mode. HPLC separation was conducted on an LC pump of Shimadzu LC-10A with SCL-10A. Sample was injected in a 10 $\mu$L volume with a PE Series 200 autosampler.

*Chromatographic conditions*

HPLC column was of Zorbax ODS 250 × 4.6 mm i.d. A constant flow rate of 0.3 mL/min was maintained and the composition of the mobile phase was programmed as follows, Solvent A: 2 mM ammonium formate in water; Solvent B: 2 mM ammonium formate in acetonitrile; gradient: 0—0.1 min, 1.0% B; 0.1—16.1 min, 1.0—20.0% B; 16.1—19.1 min, 20.0% B; 19.1—20.0 min, 20.0—1.0% B.

## Results and discussion

The formation of small peptide through the self-activation of $N$-( $O$, $O$-diisopropyl)phosphoryl amino acid (DIPP-AA) has been proved by direct FAB-MS analysis of the reaction products before, and only the $N$-phosphoryl-α-amino acid possesses such property, while the β isomer does not.[1-4] The reaction process might involve a five-membered ring penta-coordinate phosphorous intermediate, which was attacked by another molecule of free amino acid (either came from the hydrolysis of DIPP-AA itself, or from another kind of free amino acid which was added to the reaction system) to form phosphoryl peptide.[9,10]

In order to investigate the interaction between nucleoside and $N$-phosphoryl amino acid during the peptide formation reaction, $N$-( $O$, $O$-diisopropyl) phosphoryl aspartic acid (DIPP-Asp) was incubated in water-pyridine mixture in the presence of adenosine. The reaction proceeded at 40°C for 4 days and the mixture was analyzed with HPLC. Since the reactant nucleoside and formed nucleotide in the reaction system had very strong UV absorption at 254 and 210 nm, the attempt to detect the trace amount of formed peptides with UV detector at 210 nm failed. Positive ion ESI-MS provided another choice for the detection of peptide because nucleoside and nucleotide had much lower response and sensitivity to the positive ion MS mode. Under this condition, it was expected that the peptide should be detected. Indeed, Fig. 1 showed the positive ion LC-ESI-MS spectra of the reaction mixture of DIPP-Asp and adenosine.

From Fig. 1, it could be found that a strong peak was observed at about 12.54 min in the total ion current (TIC) chromatogram of the positive ion ESI-MS from the on-line HPLC, which resulted from a series of ions that might be related to each other. Therefore multi-stage MS-MS technique was used to analyze them.

Multi-stage MS-MS ( $MS^n$ ), especially tandem mass spectrometry $MS^2$, is a very useful technique in structure elucidation. In order to obtain more fragment information to confirm the structure, positive ion ESI-MS-MS was performed on the peaks of 825 and 1237 ($m/z$) from Fig. 1 (Fig. 2).

It was found from Fig. 2 that there were fragments of $m/z$ = 413, 371, 329 produced from the precursor peaks of 825 and 1237. The $m/z$ 413 $(M + H)^+$ was the protonated molecular ion of dipeptide DIPP-Asp-Asp. Hence, 825 could be proved as the dimer cluster of the dipeptide $(2M + H)^+$, since cluster ion is often observed in ESI-MS due to the intermolecular hydrogen bond formation. The same was true of the trimer cluster 1237 $(3M + H)^+$. Since the two peaks of 825 and 1237 are cluster ions of dipeptide (DIPP-Asp-Asp) which has a $m/z$ at 413 $(M + H)^+$, strong peak with $m/z$ = 413 was both observed in the positive ion ESI-MS-MS spectra of 825 and 1237. Successive loss of two isopropyl fragments ( − 42, − 42 × 2) in DIPP-Asp-Asp to $m/z$ = 371, 329, which had also been observed in the previous FAB-MS fragmentation study,[21,22] further confirmed the structure of dipeptide (Scheme 1).

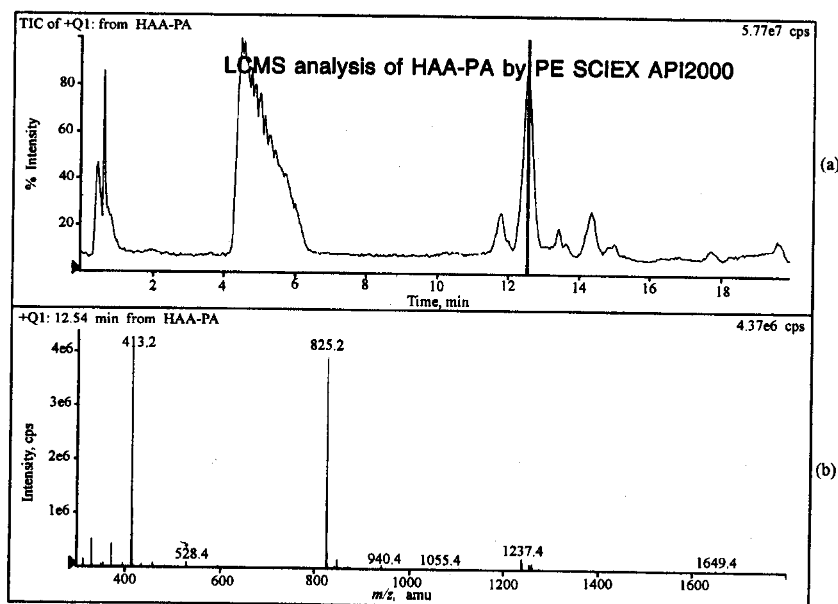


Fig. 1 LC-ESI-MS spectra of the reaction mixture of DIPP-Asp and adenosine: (a), TIC chromatogram; (b), positive ion MS spectrum of the products having a retention time of 12.54 min from the online-HPLC.

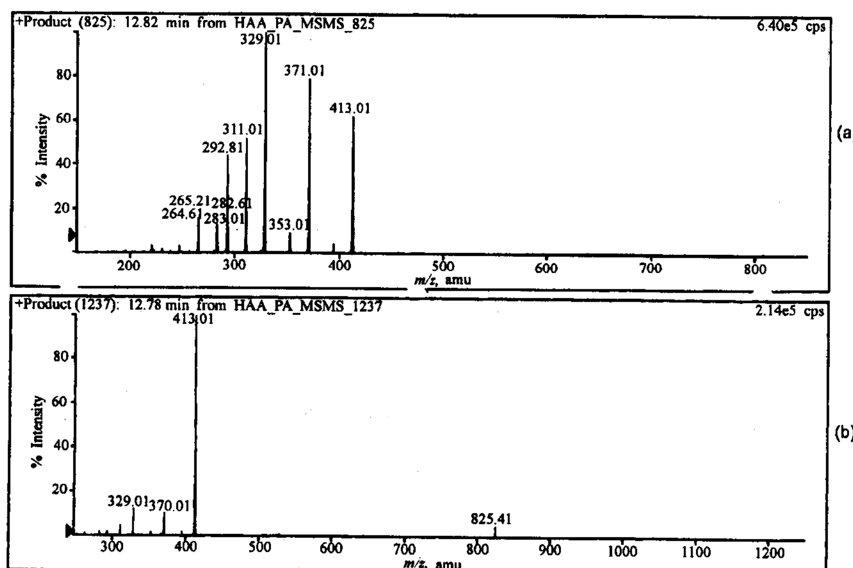


Fig. 2 Positive ion tandem mass spectroscopy of the cluster ions of (a): $m/z$ 825 and (b): $m/z$ 1237.

Therefore, the peaks of $m/z$ 413, 528, 825 and so on in Fig. 1 should be assigned as cluster ions of dipeptide (DIPP-Asp-Asp) and tripeptide (DIPP-Asp-Asp-Asp) and the cross cluster between them (Table 1).

In the same way, the formed peptides from other kinds of $N$-phosphoryl amino acids (DIPP-AA) in the presence of nucleosides were determined by the LC-ESI-MS method, and the major peptide products were showed in Table 2.

**Table 1** Cluster ions of peptides detected in the reaction system of DIPP-Asp and adenosine from Fig. 1

| Cluster ions of peptides ($MH^+$) | $m/z$ |
|---|---|
| $[(\text{DIPP-Asp-Asp}) + H]^+$ | 413 |
| $[(\text{DIPP-Asp-Asp-Asp}) + H]^+$ | 528 |
| $[(\text{DIPP-Asp-Asp})_2 + H]^+$ | 825 |
| $[(\text{DIPP-Asp-Asp} + \text{DIPP-Asp-Asp-Asp}) + H]^+$ | 940 |
| $[(\text{DIPP-Asp-Asp-Asp})_2 + H] +$ | 1055 |
| $[(\text{DIPP-Asp-Asp})_3 + H]^+$ | 1237 |
| $[(\text{DIPP-Asp-Asp})_4 + H]^+$ | 1649 |

**Scheme 1** Fragment formation pathway of cluster ion [DIPP-Asp-Asp]$_3$ in ESI-MS-MS.

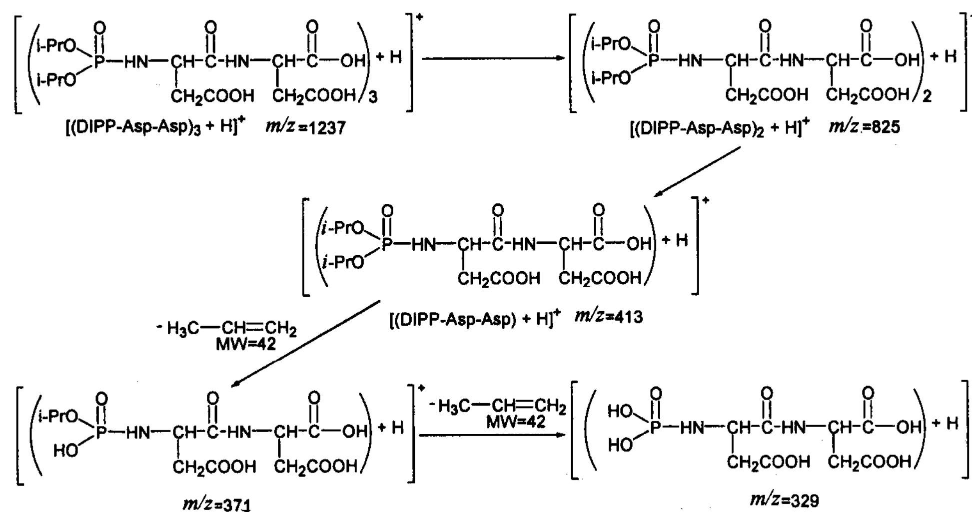


**Table 2** LC-ESI-MS data of the major peptide products in the reaction system of DIPP-AA and nucleoside

| Reaction system | Major formed peptides (MH$^+$) | $m/z$ |
|---|---|---|
| DIPP-Ser + uridine | [(DIPP-Ser-Ser) + H]$^+$ | 357 |
| DIPP-Thr + cytidine | [(DIPP-Thr-Thr) + H]$^+$ | 385 |
| DIPP-His + guanosine | [(DIPP-His-His) + H]$^+$ | 457 |

## Conclusion

It could be concluded that LC-MS-MS is a very sensitive, powerful and convenient method to study the trace amount of peptide formation reaction of DIPP-AA in the presence of nucleoside. The study of the interaction between DIPP-AA and nucleoside might provide us some clues to explain the origin of genetic code in the prebiotic chemical evolution process and give more evidence for the co-evolution theory for nucleic acid and protein. This analytical method could also be applied to study reactions when other strong UV-absorption components were presented, such as systems involving both peptide/protein and nucleotide/nucleic acid.

## References

1. Li, Y.M.; Yin, Y.W.; Zhao, Y.F., *Int. J. Pept. Protein Res.*, **39**, 375(1992).
2. Ju, Y.; Zhao, Y.F.; Sha, Y.W.; Tan, B., *Phosphorus, Sulfur Silicon Relat. Elem.*, **101**, 117(1995).
3. Zhao, Y.F.; Ju, Y.; Li, Y.M.; Wang, Q.; Yin, Y. W.; Tan, B., *Int. J. Pept. Protein Res.*, **45**, 514 (1995).
4. Fu, H.; Li, Z.L.; Zhao, Y.F.; Tu, G.Z., *J. Am. Chem. Soc.*, **121**, 291(1999).
5. Zhou, W.H.; Ju, Y.; Zhao, Y.F.; Wang, Q.G.; Luo, G.A., *Origins Life Evol. Biosphere*, **26**, 547(1996).
6. Xue, C.B.; Yin, Y.W.; Zhao, Y.F., *Tetrahedron Lett.*, **29**, 1145(1988).
7. Yan, Q.J.; Wang, Q.; Zhao, Y.F., *Phosphorus, Sulfur Silicon Relat. Elem.*, **107**, 181(1995).
8. Zhao, Y.F.; Yan, Q.J.; Wang, Q., *Int. J. Pept. Protein Res.*, **47**, 276(1996).
9. Zhao, Y.F.; Cao, P.S., *J. Biol. Phys.*, **20**, 283 (1994).
10. Zhao, Y.F.; Cao, P.S., in "*Chemical Evolution: Physics of the Origin and Evolution of Life*", Ed. Chela-Flores, J.; Raulin, F., Kluwer Academic Publishers, Netherlands, 1996, p.279.
11. Niessen, W.M.A., *Liquid Chromatography-Mass Spectrometry*, 2nd Edn., Marcel Dekker Inc., New York, 1999, p. 501.
12. Mann, M.; Meng, C.K.; Fenn, J.B., *Annal. Chem.*, **61**, 1702(1989).
13. Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M., *Mass Spectrom. Rev.*, **9**, 37(1990).
14. Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M., *Science*, **246**, 64(1989).
15. Smith, R.D.; Loo, J.A.; Edmonds, C.G.; Barinaga, C.J.; Udseth, H.R., *Anal. Chem.*, **62**, 882(1990).
16. Mann, M., *Org. Mass Spectrom.*, **25**, 575(1990).
17. Carr, S.A.; Hemling, M.E.; Bean, M.F.; Rorberts, G.D., *Anal. Chem.*, **63**, 2802(1991).
18. Smith, R.D.; Loo, J.A.; Ogorzalek Loo, R.R.; Busman, M.; Udseth, H.R., *Mass Spectrom. Rev.*, **10**, 359 (1991).
19. Ji, G.J.; Xue, C.B.; Zeng, J.N.; Li, L.P.; Chai, W.G.; Zhao, Y.F., *Synthesis*, 444(1988).
20. Li, Y.M.; Zhang, D.Q.; Zhang, H.W.; Ji, G.J.; Zhao, Y.F., *Bioorg. Chem.*, **20**, 285(1992).
21. Xue, C.B.; Yin, Y.W.; Yu, X.; Zhao, Y.F., *Org. Mass Spectrom.*, **24**, 253(1989).
22. Yin, Y.W.; Ma, Y.; Zhao, Y.F., *Org. Mass Spectrom.*, **29**, 201(1994).

(E200004081 JIANG, X.H.; LING, J.)