Asian Journal of Chemistry Vol. 22, No. 6 (2010), 4393-4397

Synthesis and Separation of L-Trytophan Oligo-Peptides Assisted by Phosphorus Oxychloride

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With the assistance of inorganic phosphorus, α-amino acids could self-assemble into a series of oligopeptides, so it could provide a new route to synthesize peptides. In this paper, the self-assembly reaction of L-trytophan mediated by phosphorus oxychloride was monitored by using ESI-MS. The proper condition of the self-assembly reaction of L-Try was reported. The reaction products were purified by RP-HPLC and the fragmentation pathway for L-Try oligo-peptides were analyzed by using ESI-MS/MS.

Key Words: Phosphorus oxychloride, L-Trp, Oligo-peptide, ESI-MS.

INTRODUCTION

With the development of studies on human genome team sequence analysis and the function protein group, the people discover that small molecular polypeptide materials which structures are relatively simple are playing the decisive role in the more and more life activity (*e.g.*, molecular recognition, signal conduction, cell differentiation and ontogenesis and so on)[1]. Recently, oligopeptides have been concerned commonly by chemists because of their strong bioactivity and activity as drug or precursor of drug[2]. In our previous work, it was found that α-amino acid could self-assemble into a series of oligopeptides with the assistance of inorganic phosphorus reagents, so it could provide a new route to synthesize peptides[3]. Recently, the chromatograph method used in polypeptides and protein separation and purification became more and more broadly[4-6]. The reversed phase high-performance liquid chromatography has the good separation effect, better resolution and high returns-ratio characteristic which widely use in separation and preparation of polypeptide[7-9]. In this paper, the reaction of L-Tryptophan self-assembly into oligopeptides mediated by $POCl_3$ was studied. The mixtures were separated and purified by RPLC and the structures of these compounds were confirmed by using ESI-MS/MS.

EXPERIMENTAL

L-Trytophan (BR) was purchased from Yuanju Biochemical Co., (Shanghai, China). Methanol (reagents for HPLC) was purchased from Tianjin Chemical

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Reagent Co., (Tianjin, China). Deionized water was generated from Milli-Q water purifying system purchased from Millipore (MA, USA). Other common chemicals and solvents were of analytical grade.

The mass spectra were obtained using a Bruker Esquire-3000 plus ion trap spectrometer equipped with a gas nebulizer probe capable of analyzing ions up to m/z 6000. The samples dissolved in methanol were ionized by electrospray ionization (ESI) and continuously infused into the ESI chamber at a flow rate of 4 μL/min. The nebulizer pressure was 15 psi and the source temperature was maintained at 300 ºC. Ions were gated into the ion trap for each scan using injection times of 200 ms. All of the experiments were acquired in positive ion mode.

Reverse-phase high performance liquid chromatography: Analyses were performed using a Agilent 1200 liquid chromatograph equipped with Agilent 1200 series binary pumps, a ultra-violet detector set at 279 nm. The column was an Agilent C_{18} (5 μm, 9.4 mm × 250 mm). Buffer A was methanol and buffer B was water. Elution was achieved by 45 % A and 55 % B. A flow rate of 1 mL/min was used throughout.

Synthetic procedure: L-Tryptophan (1 mmol) treated with $POCl_3$ (1 mmol) was added in acetonitrile, 1,4-dioxane or tetrahydrofuran (THF), stirring for 1, 2 or 6 h at different temperatures (0, 30 and 60 ºC), then quenched with water. Finally, the oligo-peptides crude products were detected by ESI-MS.

RESULTS AND DISCUSSION

In this paper, the self-assembly reaction of L-Trp mediated by phosphorus oxychloride was monitored by ESI-MS. To find the best experimental condition, the solvent, reaction time and reaction temperature were examined. Analysis of the reaction mixture by ESI-MS showed that after L-Trp had reacted with $POCl_3$ for only 1 h and quenched with water, a series of mass peaks corresponding to oligopeptides were already observed. As the reaction time prolonged, the length of peptide chain increased a little and the major product was still dipeptide. As the temperature increased, the relative intensity of dipeptide decreased. The self-assembly reaction showed good regularity in acetonitrile and THF, but the latter was better than the former. However, the oligopeptides were not found in 1,4-dioxane. The result showed that the proper condition of synthesis was the mole ratio of L-Trp and $POCl_3$ of 1:1, stirring at 30 ºC for 1 h in THF.

There are many methods, such as ion exchange chromatography and reversed-phase high performance liquid chromatography[10] (RPLC) for separation of amino acid and oligopeptide. By reversed-phase ion pair HPLC-ESI-MS/MS, the amino acid, Trp-Trp dipeptide and cyclo-Trp-Trp dipeptide were separated and quantified. Through several experiments, the methanol/water system was selected to purify the oligo-peptides mixture. Then ESI-MS in positive ion mode was selected to detect and identify the molecular masses of the effluent from the RP-HPLC. A series of mass peaks corresponding to oligo-peptides of L-Trp were observed. The

results showed that the mobile phase, which be chosen were suitable in the purification of L-Trp oligo-peptides.

The RP-HPLC and ESI-MS spectra of L-Trp oligo-peptides are shown in Figs. 1 and 2. The peak eluted at 12.416, 18.221 and 25.170 min, whose m/z was at 205,

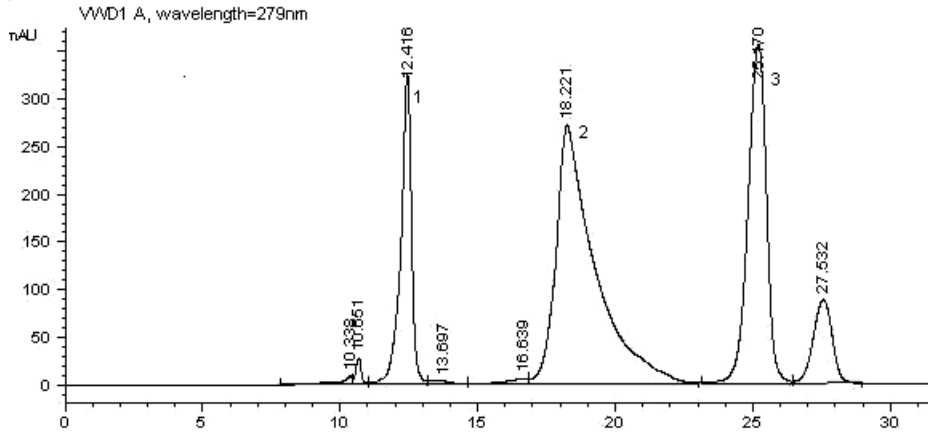

Fig. 1. HPLC spectra of reaction mixture

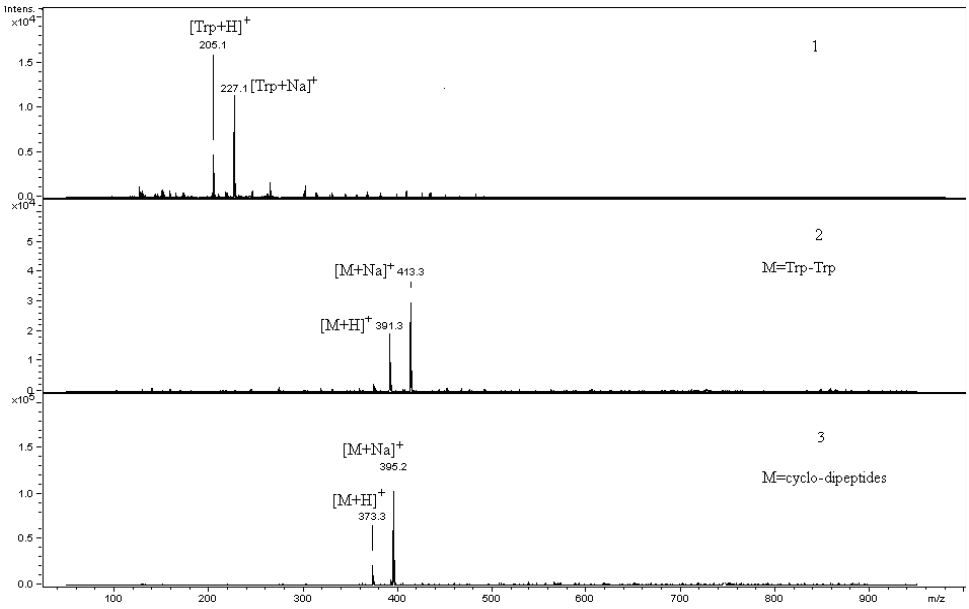

Fig. 2. Positive ESI-MS spectra of separation of each component

391 and 373, were identified as amino acid monomer, Trp-Trp dipeptide and cyclo-Trp-Trp dipeptide, respectively. The contents of dipeptides were calculated by area per cent method. The concentration of Trp-Trp was about 46.2 % and the concentration of cyclo-Trp-Trp was about 28.7 %. Their structures were further identified

by ESI-MS/MS and the fragmentation pathways were investigated. Take the Trp-Trp for example, the ion at m/z 205 came from the loss of L-Trp residue. The formation of the ion at m/z 374 occur the Trp-Trp dipeptide which lost NH_3, then lost CO corresponding to the ion at m/z 296. The fragmentation pathway was shown in Figs. 3 and 4. The corresponding ESI-MS/MS data for L-Trp oligo-peptides are shown in Table-1.

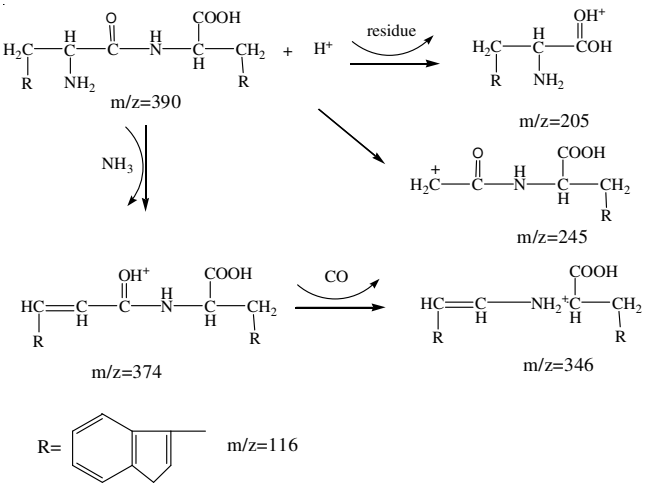

Fig. 3. Fragmentation pathway of Trp-Trp dipeptide

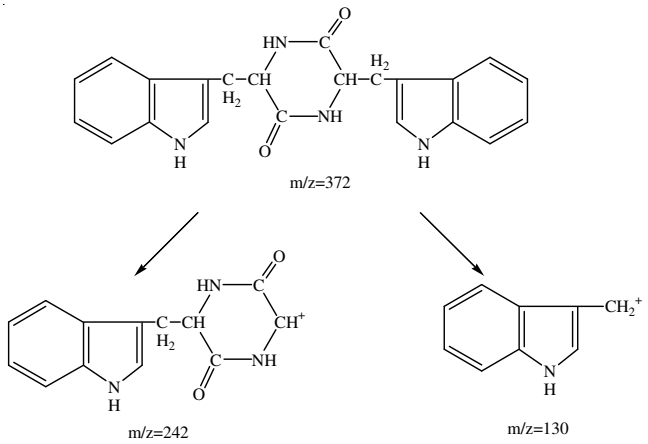

Fig. 4. Fragmentation pathway of cyclo-Trp-Trp dipeptide

TABLE-1
POSITIVE ION ESI-MS/MS DATA FOR DIPEPTIDE

Compounds	Precursor ions $[M + H]^+$	Fragment ion (relative intensity, %)
Dipeptide	391 (30)	374 (100) 346 (8) 245 (30) 205 (8)
Cyclo-dipeptide	373 (10)	130 (100) 242 (90)

Conclusion

The reaction of self-assembly into oligo-peptides of L-trytophan mediated by phosphorus oxychloride was studied. Compared different experimental conditions, the proper condition of synthesis was the molar ratio of L-Trp and $POCl_3$ of 1:1, stirring at 30 ºC for 1 h and in THF. With the assistance of HPLC-ESI-MS, the products from the reaction were separated and identified. This method to synthesize dipeptide can be improved to get reasonable fields through further study.

ACKNOWLEDGEMENTS

The authors would like to thank the financial supports from the Chinese National Science Foundation (No. 20572016, 20772023), Henan Province Science Foundation for Prominent Youth (No.0312000900) and Office of Education of Henan Province (No.2006KYCX017).

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(*Received*: 31 July 2009; *Accepted*: 12 February 2010) AJC-8420