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Influential Factor of Temperature on Synthesis of L-Arginine Oligo-Peptides Mediated by Phosphorus Oxychloride

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Electrospray ionization mass spectrometry (ESI-MS) is one of the most widely applicable bio-MS technologies, with the characteristics of no fragmentation. Electrospray ionization mass spectrometry has proven to be an extremely powerful technique for the analysis of involatile, polar and thermally labile compounds which exist as ions (positive and negative) in solution. In this paper the effect of temperature on L-arginine self-assemble into oligo-peptides which analyzed by ESI-MS mediated by phosphorus oxychloride was investigated. The structure of Arg-Arg dipeptide was confirmed and the fragmentation pathway of Arg-Arg dipeptide was analyzed by ESI-MS/MS.

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

INTRODUCTION

Oligopeptides have been widely used to identify and examine the specificities of immune system[1] and for screening bioactive ligands that bind to DNA and enzymes[2] and are recognized as powerful tools for obtaining detailed information about α-protein-protein interactions[3]. There are numerous methods for constructing peptide libraries such as multipin synthesis[4], tea-bag method[5], split synthesis[6] and light directed synthesis[7]. Electrospray ionization mass spectrometry (ESI-MS) has been widely used in analysis of poly-peptide, protein and biological macro-molecule[8]. Generally, it commonly uses the y type ion or the b type ion to determine the sequence of polypeptides with the mass spectra, then extract amino acid residue quality according to neighbour ionic mass difference, at last obtain the sequence of polypeptides[9-11]. Though, it is intricacy to polypeptide fragmentation pathway at mass spectrum, sometimes it could not get integrated y type ion or the b type ion[12]. In this paper, the effect of temperature on self-assembly of L-Arg-peptides mediated by phosphorus oxychloride was investigated by using ESI-MS.

EXPERIMENTAL

L-Arginine (BR) was purchased from Yuanju Biochemical Co. (Shanghai, China). Methanol (Reagents for HPLC) was purchased from Tianjin Chemical Reagent Co. (Tianjin, China). Deionized water was generated from Milli-Q water purifying system purchased from Millipore (MA, USA). Other chemicals and solvents were of analytical grade.

Mass spectrometry: Mass spectra were acquired 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.

Synthesis: L-Arg (1.0 mmol) and $POCl_3$ (0.092 mL) were taken in acetonitrile, with stirring at different temperature for 1 h, then sampled and quenched with H_2O (15 mL).

RESULTS AND DISCUSSION

The L-arginine was treated with $POCl_3$ in acetonitrile, stirring for 1 h at different temperature, then quenched with H_2O (15 mL). Analysis of the reaction mixture by ESI-MS showed that after L-arginine had reacted with $POCl_3$ for only 1 h and quenched with water, a series of mass peaks corresponding to oligo-peptides were already observed. With the improvement of reaction temperature, the velocity of L-arginine self-assembly into oligopeptides did not increase but decrease. The dipeptide was observed by ESI-MS with stirring for 1 h at 30 ºC (Fig. 1). Also, a little dipeptide, tripeptide and tetrapeptide were found at 40 ºC and the relative intensity of dipeptide obviously decreased. When temperature was increased to 50 ºC, the oligopeptide were not observed. One important

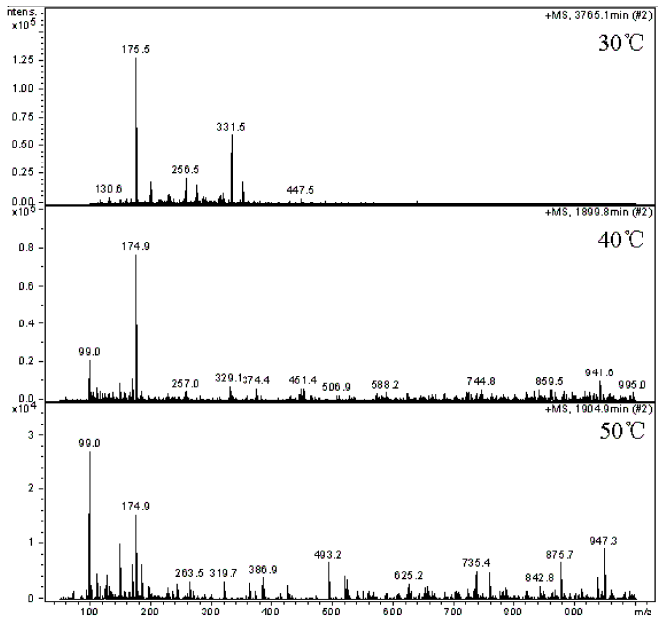

Fig. 1. Positive ESI-MS spectrum of L-Arg oligo-peptides

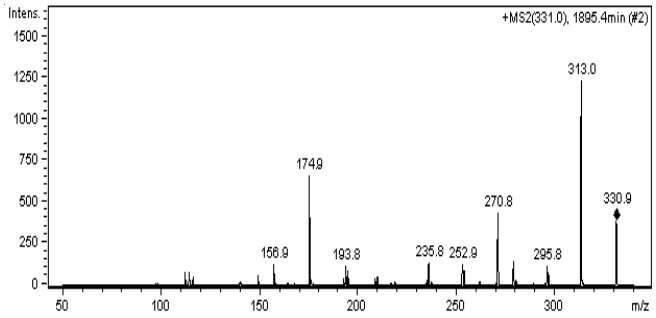

Fig. 2. ESI-MS/MS of Arg-Arg dipeptide

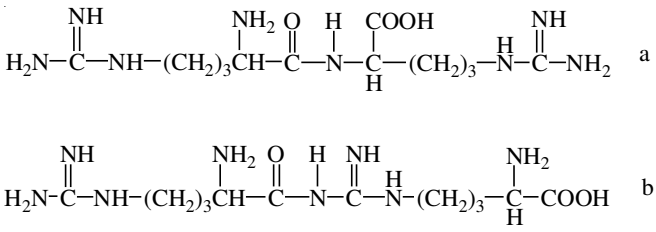

Fig. 3. Two structures of Arg-Arg dipeptide

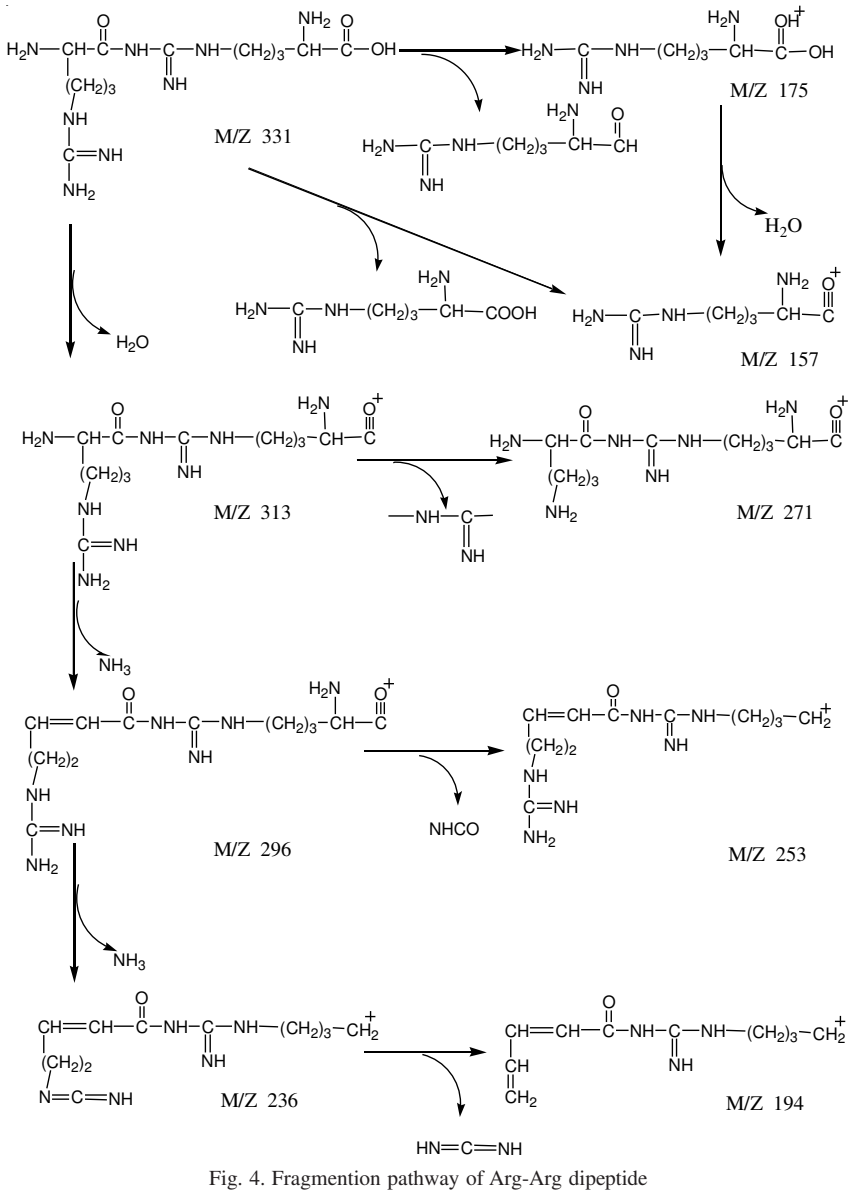

Fig. 4. Fragmention pathway of Arg-Arg dipeptide

characteristic of arginine is that its side chain end contains a guanidyl. The ability of guanidyl forming hydrogen bond is more than amido as well as its directionality and stability. Therefore, with the improvement of reaction temperature, molecular motions intensified and the molecule polymerized is much easier. The result showed that the proper condition of self-assembly reaction was stirring at 30 ºC.

In order to extend this methodology, the MS/MS spectra of Arg-Arg dipeptide was recorded (Fig. 2). Fragmentation pathway was shown in Fig. 4. The molecule ion at m/z 331 in the MS/MS spectra of the $[M+H]^+$ of compound was identified as Arg-Arg dipeptide. The ion m/z 331 of dipeptide produced fragment ion m/z 175 by the b cleavage. The formation of the ions at m/z 313 occurs the dipeptide which lost H_2O, then lost NH_3 corresponding to the ion at m/z 296. The ions observed at m/z 236 came from the cleavage of ion m/z 296 which also lost NH_3. The two different structures of dipeptide were showed in Fig. 3. From fragmentation pathway, we deduced that compound b showed in Fig. 3 was the proper structure.

Conclusion

With the improvement of reaction temperature, the velocity of L-Arg self-assembly into oligopeptides did not increase but decrease. The proper condition of self-assembly reaction was stirring at 30 ºC. The Arg-Arg dipeptide's structure [Fig. 3(b)] was identified by ESI-MS/MS and the fragmentation pathway of Arg-Arg dipeptide was proposed.

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