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ESI/MS of N-(*O, O*-Diisopropyl) Phosphoryl Aromatic Amino Acids and their Stability Investigation Using HPLC/UV/ESI/MS

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Abstract: The full scan ESI/MS and ESI/MS^2 of N-(*O, O*-diisopropyl) phosphoryl aromatic amino acids (DIPPAAAs), N-(*O, O*-diisopropyl) phosphoryl phenylalanine, N-(*O, O*-diisopropyl) phosphoryl tryptophan and N-(*O, O*-diisopropyl) phosphoryl tyrosine, were obtained. The specific ions for them were found. Their stability in the LC mobile phase was investigated using developed HPLC/UV/ESI/MS and the results demonstrated that the DIPPAAAs were stable in the mobile phase (5 mmol/L NH_4Ac-MeCN (80:20,v/v, pH7.5) within 48 h.

Keywords: ESI/MS, HPLC, diisopropyl phosphorylation, amino acid.

In the past work, it has been found that N-(*O, O*-diisopropyl) phosphoryl amino acids (DIPPAAs) took place some interesting biomimicking reactions. DIPPAA can also react with nucleosides to form nucleic acids[1,2]. These proved that DIPPAA as intermediate might have played an important role in prebiotic synthesis of nucleic acids and proteins during origins of life[3]. In the course of reaction, its stability directly influences the reaction progress and the products composition, which might lead to the different reaction mechanism. Therefore, the use of high-purity DIPPAA and keeping its high stability in the reaction system can simplify the reaction and ensure the correct information of the products. Thus, it is necessary to develop a novel approach to track its purity and stability. To date, some approaches, *e.g.* spectrometry[4,5] and capillary electrophoresis[6] have been developed for analysis of some DIPPAAs. However, HPLC technique with the advantages of rapidity, precision, accuracy and automation has not been extensively applied for their analysis. In this paper, three aromatic DIPPAAs(DIPPAAAs), N-(*O, O*-diisopropyl) phosphoryl phenylalanine(DIPPPhe), N-(*O, O*-diisopropyl) phosphoryl tryptophan(DIPPTrp) and N-(*O, O*-diisopropyl) phosphoryl tyrosine(DIPPTyr), were chosen as model DIPPAAs. Their ESI/MS and ESI/MS^2 were obtained, and a rapid HPLC/UV/ESI/MS was successfully developed to examine their stability in the mobile phase.

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Experimental

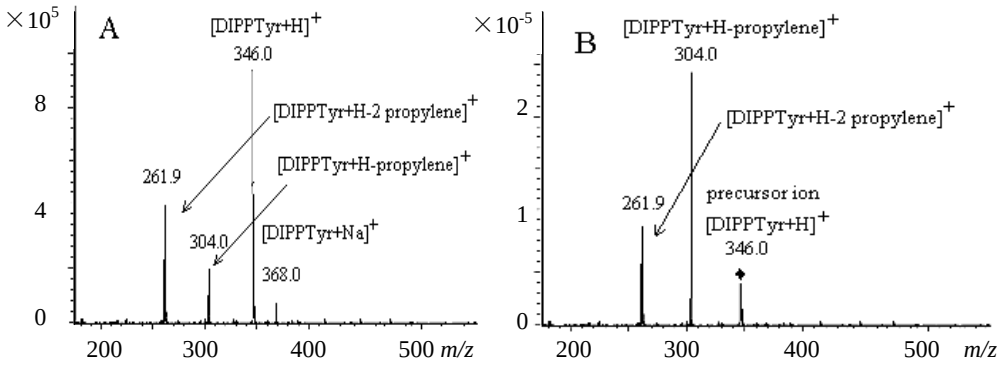
The HPLC-MS was performed on an Agilent 1100 series HPLC (Agilent Co., Germany) and an Esquire 3000 ESI/MS with ion trap mass spectrometer (Bruker Daltonik Gmbh, Germany). The separation was carried out on a Supelco LC-18 column (250×4.6 mm) with mobile phase of 5 mmol/L NH_4Ac-MeCN (80:20, pH7.5) at a flow-rate of 0.8 mL/min, and the detection wavelength was at 230 nm. The injection volume was 20 μL. The full scan ESI/MS were obtained with both negative and positive polarity. The ion source temperature was 300 °C. The capillary voltage was ± 4.0 kV.

DIPPPhe, DIPPTrp and DIPPTyr products were prepared according to our previous description[7] with methanol-ether recrystallization for 3 times. Three DIPPAAAs were characterized using FTIR, NMR and MS, and the content was more than 99.0% (using the area normalization method of HPLC). All solvents and sample solutions used for HPLC/UV/ESI/MS were filtered through a 0.45 μm membrane.

Results and Discussion

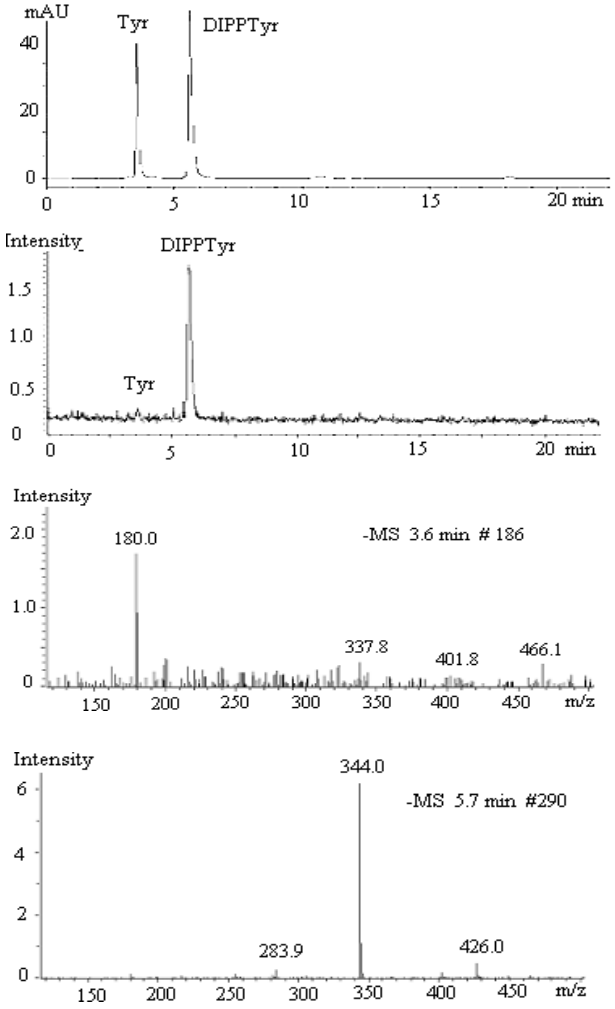
The base peak and the most abundant ions (with the relative abundance) of the ESI/MS and ESI/MS^2 of DIPPPhe, DIPPTrp and DIPPTyr under both positive and negative polarity were obtained. The base peaks of DIPPPhe, DIPPTrp and DIPPTyr were their corresponding protonated or deprotonated molecule. The similar cleavage pathways were observed through successive losses of one and two-molecular of propylene under the positive ESI, and with losses of one-molecular propylene and one-molecular isopropanol under the negative polarity. As an example, **Figure 1** showed +ESI/MS and $+ESI/MS^2$ for DIPPTyr. These MS and MS^2 data could provide two alternative strategies to characterize DIPPAAAs. However, the negative mode produced comparatively less fragments and a higher intensity of the signals than in the positive polarity. Therefore, the negative polarity is obviously superior and the molecular ions $[M-H]^-$ at *m/z* 328, 367 and 344 were chosen as the specific ions for identifying DIPPPhe, DIPPTrp and DIPPTyr in the samples, respectively.

Figure 1 ESI/MS (A) and ESI/MS2 of DIPPTyr under positive polarity



As DIPPAAs from the reaction with phosphorylation regent and corresponding amino acids (AAs), the possible impurities are their corresponding AAs. Thus, how to optimize LC separation of DIPPAA and AA is becoming an important task. By comparing the retention time and resolution on different columns (NH_2, C_8 and C_{18}) under the different elution modes (isocratic or gradient elution) with different mobile phases, the reasonable retention time and optimal separation of DIPPAAs and AAs were obtained on the column of C_{18} with the mobile phase of 5 mmol/L NH_4Ac-MeCN (80:20,v/v) at 0.8 mL/min. More importantly, the DIPPAAs showed the more stable in the mobile phase with NH_4Ac than in the others without NH_4Ac. Taking analysis time,

Figure 2 HPLC/UV/ESI/MS of DIPPTyr placed in the mobile phase for 96 h



From the upper to bottom: UV chromatogram, TIC chromatogram, ESI/MS extracting at 3.5 min and at 5.7 min in UV chromatogram.

peak shape, resolution between DIPPAA and AA into consideration, the isocratic elution with the mobile phase of 5 mmol/L NH_4Ac-MeCN (80:20) at 0.8 mL/min and Supelco LC-18 were chosen as the optimal mobile phase and column.

Under the proposed HPLC conditions with the concentration range of 1-100μg/mL at 230 nm, the regressions between peak area (A) and concentration (C, μg/mL) yielded the following equations: for DIPPPhe, A=17704 C- 1150 (n=6,R^2=0.9998); for DIPPTrp, A=21704 C- 1924 (n=6,R^2=0.9999); for DIPPTyr, A=27904 C-1442 (n=6,R^2=0.9995). The limits of detection (LODs) (S/N=2) were 0.50, 0.40 and 0.30 μg/mL, and the recoveries (n=3) were 99.25%, 97.10% and 102.37% for DIPPPhe, DIPPTrp and DIPPTyr, respectively. The RSDs of the peak areas and retention times were 1.2% and 1.4% for DIPPPhe, 1.5% and 1.1% for DIPPTrp, 1.3% and 1.9% for DIPPTyr, respectively.

To examine the stability of DIPPAAA in the mobile phase, the HPLC/UV/ESI/MS of the real samples was carried out every 12 h for 4 days. The results showed that, within 48 h, the peak areas for each DIPPPhe, DIPPTrp and DIPPTyr remained unchangeable (RSDs<2.5%). No distinctive corresponding AAs were found. After 48 h, DIPPAAAs eventually degraded and finally Trp, Phe and Tyr present in the spectra, which were characterized with their specific ions $[M-H]^-$, at *m/z* 203 for Trp, at *m/z* 164 for Phe and at *m/z* 180 for Tyr, respectively. **Figure 2** (one example) demonstrated the spectra of DIPPTyr after being placed in the mobile phase for 96 h. Besides the corresponding AAs, some other unknown peaks were also present due to dephosphorylation effect.

ESI/MS and ESI/MS^2 provided the cleavage pathway and specific ions of DIPPPhe, DIPPTrp and DIPPTyr, and HPLC/UV/ESI/MS has been developed for their analysis. The high stability of DIPPAAAs in the mobile phase implied that NH_4Ac-MeCN would be suitable for using as a reaction solvent system.

Acknowledgments

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