Comparison of collision-induced dissociation and electron-induced dissociation of Phillyrin using FT-ICR MS

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Abstract

Electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry using collision-induced dissociation (CID) and electron capture dissociation (ECD) at high mass resolution was first applied to investigate the characteristic fragment ions of phillyrin. The CID experimental results demonstrated the elemental composition of fragment ions unambiguously, so a reasonable fragmentation pathway of phillyrin was proposed. The ECD fragmentation mechanism was believed to be fundamentally different from the CID method. ECD could be used not only in the biological field but also as a powerful complement to the structural identification of small molecular compounds. The characteristic fragmentation pathways were helpful in analyzing and interpreting the stability and property of the parent ion.

Key word: Phillyrin, FT-ICR MS, MS/MS, CID, ECD

1. Introduction

Oleaceae forsythiae fructus is a traditional herbal medicine containing triterpenoids, lignans, and glycosides. It is used as an anti-inflammatory, diuretic, antidote, and antibacterial medicine in some Asian countries because of its pharmacognostic properties. Phillyrin is a class of natural glycosidic lignan compounds with a bis-tetrahydrofuran ring [1]. As the main effective composition of forsythiae fructus, phillyrin is separated or extracted from forsythiae fructus by different methods [2-4].

Numerous studies have reported that phillyrin possesses excellent pharmaceutical properties, such as antibacterial, antiviral, anti-inflammatory, antipyretic, hepatoprotective, cardiac diuretic, antiemetic, and antihypertensive activities. Zhao demonstrated the anti-obesity effect of phillyrin in nutritive obesity mice through indices of fat wet weight, fat index, number of fat cells per unit visual field, Lee's index, jejunum microvillus area, serum triglyceride, and cholesterol [5].

Do MT observed phillyrin to strongly inhibit high glucose-induced fatty acid synthase expression by modulating the activation of sterol regulatory element-binding protein 1c [6]. Díaz Lanza AM revealed that phillyrin exerts a preferential effect on the cyclo-oxygenase pathway by inhibiting the release of the cyclo-oxygenase metabolite prostaglandin E2 and, to a lesser extent, reducing thromboxane B2 levels [7]. Kong showed that phillyrin partially restores insulin-stimulated 2-DOG uptake, which was reduced by tumor necrosis factor- α , with concomitant restoration in the serine phosphorylation of insulin receptor substrate-1 and

insulin-stimulated Glut₄ translocation to the plasma membrane [8].

Few reports focused on the spectral information of phillyrin, especially mass spectrometry (MS). MS plays a crucial role in the structural elucidation of effective substances from traditional medicine. Secure interpretation of the MS of each compound requires an adequate understanding of the key fragmentation process of the mass spectrum. In this work, an alternative tandem MS (MS/MS) method was recently developed, in which isoaspartic residues in peptides and proteins were unambiguously differentiated and quantified by electron capture dissociation (ECD) [9-11]. ECD was first introduced by Zubarev and has since been widely implemented for the routine structural analysis of biological molecules [12, 13]. ECD of peptide ions provides fragments complementary to those generated by other MS/MS methods [14, 15]. The principle of ECD involves the direct use of energy gain upon electron capture to cleave the N–CR bond rather than become randomized along the molecule to break the weakest bond [16-18]. Multiple charged gas-phase molecular ions produced by electrospray ionization (ESI) traditionally react with low-energy electrons in Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), which was recently implemented in a radiofrequency ion trap [19-24]. The structure of phillyrin was shown in Fig. 1. The present study was the first to report on the fragmentation pattern of phillyrin in ESI FT-ICR MS by collision-induced dissociation (CID) and ECD.

2. Experiments

Mass spectrometry analyses were performed on Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (BrukerDaltonics, Bremen, Germany) with 7 T magnet (Magnex, UK) and electrospray ionization source (Apollo II, BrukerDaltonics, Bremen, Germany). The conditions employed for ESI source were a drying gas temperature of 180°C, a nebulizing gas pressure of 1.0 L/min, with 4.0 kV on the atmospheric side of the glass capillary, 3.5 kV on the atmospheric chamber end cap shield, and the capillary exit voltage was kept 36 V to avoid capillary-skimmer dissociation in the ESI interface. All ion excitations were performed in broad band mode (frequency sweep radial ion excitation). The base pressure in the ICR vacuum chamber was 4.0 e-11 mbar and in the quadrupole region 6.0 e-6 mbar. For CID experiments, argon was used as the collision gas and the collision energy was set within a certain range. The following ECD parameters were employed: electron irradiation time, 35-100 ms; cathode potential, -0.2 to -1.2 V. Phillyrin (10 ppm) in methanol/water (v/v, 1:1) were injected directly to the ionization source by a microliter pump at a flow rate of 2.0 µL/min.

3. Results and discussion

The positive ion mode ESI mass spectra of phillyrin are shown in Fig. 2, and the corresponding fragmentation pathways are displayed in Scheme 1. The spectrum (Fig. 2) was relatively clean. The quasi-molecular ion peak $[M+Na]^+$ at $m/z = 557.1994$ was dominant compared with the theoretical value of 557.1993, with a difference of only 0.2 ppm. A minor peak at $m/z = 573.1734$, which corresponded to $[M+K]^+$, was also observed. This peak yielded a difference of only 0.17 ppm when compared with the theoretical value of 573.1733. Fig. 3 shows the ESI FT-ICR MS by CID fragmentation spectra of $[M+Na]^+$ ion at $m/z 557.1994$. The precursor ion $[M+Na]^+$ was selected and subsequently fragmented in MS/MS mode to obtain the product ion spectra yielding useful structural information. The fragment ions were produced as the prominent product ions at $m/z 309.0942$ because of a loss of $C_{14}H_{16}O_4$, whereas $m/z 379.1150$ was

$[\text{C}_{20}\text{H}_{20}\text{O}_6+\text{Na}]^+$ by a loss of $\text{C}_7\text{H}_{14}\text{O}_5$ through the formation of the peroxide bond. For MS/MS operation, ion spectra were generated at 27 eV, which indicated that the compound was relatively stable and required a high energy to break.

To understand the stability of the structure, the same sample was subjected to negative ionization, and considerable information was summarized in Fig. 4 and Fig.5. A major ion was observed at m/z 533.2028, which corresponded to the deprotonated molecule $[\text{M}-\text{H}]^-$. Notably, several peaks in Fig.5 remained ambiguous because of trace impurities in the sample.

The possible cleavage of specific rules was discussed in Scheme 2. An obvious peak was located at m/z 371.1497 under the condition of isolate without addition of any collision energy. The product ion spectra were obtained at 4.5 eV by CID to minimize fragmentation. A very strong peak was conspicuous at m/z 356.1262 obtained from m/z 371.1497 by loss of a methyl group.

ECD was adopted for investigation to understand further the cleavage of phillyrin. Fig. 5 displays the results of ESI FT-ICR MS/MS analysis of $[\text{M}-\text{H}]^-$ in the negative ion mode by ECD. However, in the negative ion mode, several peaks were not found in CID. The corresponding cleavage pathway was presented in Scheme 3. No special difference was found in the positive ion mode because of the basic principle of ECD. An isolated ion was observed at m/z 533.2028, which corresponded to the deprotonated molecule $[\text{M}-\text{H}]^-$ and produced a characteristic fragment ion at m/z 519.1898 by the loss of CH_3 . In addition, fragment ions at m/z 483.1659 and m/z 439.1398 were observed by further molecular rearrangement. The corresponding ESI-MS/MS mass spectra of the compound and the fragment ions are summarized in Scheme 4. Interestingly, when m/z 533.2028 was isolated, a relatively strong peak at m/z 555.1849 was visible, i.e., $[\text{M}+\text{Na}-2\text{H}]^-$. Compared with the theoretical value of m/z 555.1848, the error was only 0.18 ppm. Whether in the CID or ECD mode, the peaks at m/z 455.17 and m/z 371.1500 were present because of the same crack bonds, whereas some minor differences must be noted, in which the precursor m/z 371.1500 ion produced m/z 357.1359 because of the basic of ECD. ECD may have acquired an electron with the random fracture of the bond for low energy, thereby resulting in weak peaks. Further studies that aim to exploit the fragmentation pathways of other medicinal compounds are currently underway.

4. Conclusion

The logical fragmentation pathways of phillyrin by CID and ECD were proposed through ESI FT-ICR MS. These pathways are important for the rapid identification of phillyrin compounds. The molecular weight and the characteristic fragment ions obtained by MS/MS were consistent with the basic molecular structure. This study extended the application scope of FT-ICR MS and offered a new means to investigate the reaction mechanism of Oleaceae. However, the results of this experiment provided a needed baseline for future studies. ECD could be used not only for the biological field but also as a powerful complement in the structural identification of small molecules. Further studies on structural analysis and combining the developed technique with ultrahigh resolution MS would present a powerful tool for the analysis of small molecules (<1000 Da).

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Fig. 1. The structure of phillyrin.

Fig.2. ESI FT-ICR MS analysis of Phillyrin in positive ion mode.

Figure(s)

Fig.3. ESI FT-ICR MS/MS analysis of $[M+Na]^+$ in positive ion mode by CID.

Fig. 4. ESI FT-ICR MS/MS analysis of $[M-H]^-$ in negative ion mode by CID.

Fig. 5. ESI FT-ICR MS/MS analysis of $[M-H]^-$ in negative ion mode by ECD.

Figure(s)

Scheme 1. The possible fragmentation pathways of $[M+Na]^{+}$ ion in positive mode by ESI FT-ICR MS/MS CID.

Figure(s)

Scheme 3. The possible fragmentation pathways of quasi-molecular ion in negative mode by ESI FT-ICR MS/MS ECD.

Figure(s)

Scheme 2. The possible fragmentation pathways of $[M-H]^-$ ion in negative mode by ESI FT-ICR MS/MS CID.

Fig. 4. ESI FT-ICR MS/MS analysis of $[M-H]^-$ in negative ion mode by CID.

Fig. 5. ESI FT-ICR MS/MS analysis of $[M-H]^-$ in negative ion mode by ECD.