Analysis of Functional Groups of Porphyrins by Collision-Induced Dissociation Mass Spectrometry/Mass Spectrometry

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Collision-induced dissociation mass spectrometry/mass spectrometry (CID-MS/MS) was effective for estimating the substituent groups of porphyrin. The substituents of the porphyrin plane, which form the pathway for an oxygen-binding reaction, are essential for its oxygen-binding affinity. The CID-MS/MS of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt and 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper was measured, and their fragmentations were studied using *B/E*-constant and $B(1-E)^{1/2}/E$ -constant linked-scan techniques. These CID-MS/MS spectra clearly show that the substituent group represents the eliminations of the alkyl and acyl radicals from the molecular ions. Thus, the CID-MS/MS technique has been proven to be useful for characterizing porphyrin substituents.

Keywords Porphyrin, mass spectrometry, collision-induced dissociation mass spectrometry/mass spectrometry, *B/E*-constant linked-scan, $B(1-E)^{1/2}/E$ -constant linked-scan

A number of porphyrin derivatives with specific and reversible oxygen-binding abilities have been synthesized by mimicking hemoglobin and myoglobin. The oxygen-binding affinity of these porphyrins are strongly dependent on the species of the central metals, the base ligands at the fifth site, the porphyrin structure, and the surrounding environment. The substituents of the porphyrin plane, which form the pathway for the oxygen-binding reaction, affect the oxygen-binding affinity.^{1,2}

An analysis of the structure of the functional groups of the porphyrins is generally carried out by infrared (IR) and (NMR) spectroscopy. There are difficulties, however, in the structural studies of porphyrins by IR and NMR spectrometry: NMR spectrometry requires a large sample size (0.1 mg), and IR spectrometry cannot distinguish between a substituent group in number, that is, whether there are three substitution sites or four. The technique of collision-induced dissociation mass spectrometry/mass spectrometry (CID-MS/MS) with the linked-scan techniques has been reported by many researchers.³⁻¹⁰ However, there has been no report concerning analyses of the structure of the functional groups of the porphyrins.

In order to obtain information about the substituent functional groups of the porphyrin plane, the losses from the porphyrin molecular ions were analyzed by CID-MS/MS with the linked-scan techniques for cases in which the substituent groups were identical.

Experimental

All of the chemicals used were commercial of analytical grade, and were used without further purification.

5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt and 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper were prepared according to a procedure of Collman *et al.*¹¹ Figure 1 shows the chemical structures of these compounds. Figures 2 and 3 show the EIMS spectra of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt and 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper.

All of the experiments were performed using a double-focusing reversed-geometry mass spectrometer (JEOL JMS-SX102A).

The magnetic and electric fields were calibrated against Perfluorokerosene Super-high boiling (Tokyo Kasei Kogyo Co).

The electron ionization (EI) conditions were as follows: ionizing voltage, 70 V; ion source temperature, 250°C; ion acceleration voltage, 10 kV; post acceleration detector voltage, 10 kV. Direct inlet system was used. The mass spectrometer was equipped with a data system (JMS-complement), and full mass spectra (0–2000 u) were recorded every 10 s. 5,10,15-Tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt and 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper, re-

Fig. 1 Chemical structures of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt (a) and 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper (b).

Fig. 2 EIMS spectrum of the 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt.

Fig. 3 EIMS spectrum of 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper.

spectively, were applied directly to the sample holder of the direct inlet system. The temperature of the sample holder was raised to 480°C according to the program. Molecular ions were selected as precursor ions for CID-MS/MS studies. Helium gas was introduced in order until the intensity of the molecular ions was decreased to

50%, and the B/E -constant linked-scans and $B(1-E)^{1/2}/E$ -constant linked-scans were recorded.

Results and Discussion

CID- B/E -constant linked-scan spectrum of the 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt of molecular ion (m/z 983) produced product ions at m/z 898, 841, 813, 756 and 728. The spectrum is shown in Fig. 4.

Figure 5 shows the CID- $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss of 85 u) of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt. The neutral loss spectrum (Fig. 5) shows that a neutral species of 85 u, corresponding to the C_5H_9O radical, is eliminated not only from the molecular ion (m/z 983), but also from the fragment ions at m/z 898 and 813, giving product ions at m/z 898 and 813, respectively. The absence of fragment ions at m/z 756, which are present in Fig. 4, indicates that the elimination of C_5H_9O also occurs from the ion at m/z 841, which is possibly derived from an other series of fragmentation.

Figure 6 shows the CID- $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss of 57 u) of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt. Based on these spectral data (Figs. 4–6), the presence of three amidophenyl groups (pivaloyl C_5H_9O 85 u) is concluded. Similarly, the CID- B/E -constant linked-scan spectrum of the 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper of molecular ion (m/z 1071) produced product ions at m/z 986, 929, 901, 844, 816, 759 and 731, which indicates, again, a series of eliminations of C_5H_9O (Fig. 7).

Figure 8 shows the CID- $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss, 85 u) of 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper. Figure 9 shows the CID- $B(1-E)^{1/2}/E$ -constant linked-scan spec-

Fig. 4 Collision-induced dissociation B/E -constant linked-scan spectrum of the 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt of molecular ion (m/z 983).

Fig. 5 Collision-induced dissociation $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss of 85 u) of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt.

Fig. 6 Collision-induced dissociation $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss of 57 u) of 5,10,15-tris(*o*-pivalamidophenyl)monoaminoporphyrinatocobalt.

trum (neutral loss 57 u) of 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper. Based on these spectral data (Figs. 7–9), the presence of the four amidophenyl groups (pivaloyl C_5H_9O 85 u) is elucidated.

In conclusion, collision-induced dissociation mass spectrometry/mass spectrometry (CID-MS/MS) is useful for analyzing the modification structures of porphyrins.

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Fig. 7 Collision-induced dissociation B/E -constant linked-scan spectrum of the 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper of molecular ion (m/z 1071).

Fig. 8 Collision-induced dissociation $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss of 85 u) of 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper.

Fig. 9 Collision-induced dissociation $B(1-E)^{1/2}/E$ -constant linked-scan spectrum (neutral loss of 57 u) of 5,10,15,20-tetrakis(*o*-pivalamidophenyl)porphyrinatocopper.

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