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Sequence and modified group analysis on C-terminal modified analogs of endomorphin-2 using electrospray ionization-Fourier transform ion cyclotron resonance mass spectrometer

ZHU ChuanJun[1], HU ChangFeng[1], SHAO Xuan[1,2], YAO JinLong[2], WANG ChangLin[2], WANG Rui[2†] & CUI YuXin[1†]

[1] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Science, Peking University, Beijing 100083, China;
[2] Institution of Biochemistry and Molecular Biology, School of Life Science, Lanzhou University, Lanzhou 730000, China

In this paper, a series of C-terminal modified analogs of endomorphin-2 is investigated using ESI-FT-ICR-MS. Some b, y″, a, and internal ions are found in the CID spectra and slight mass differences between the calculated and observed results are obtained. Moreover, if the C-terminal modified group is t-butyloxy, it can lose butene through McLafferty rearrangement. FT-ICR MS shows its power in peptide sequencing successfully helping us obtain the structure of peptide analogs.

endomorphin-2, opioid peptide analogs, ESI, FT-ICR MS, McLafferty rearrangement

Endomorphin-1 (EM-1: Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2: Tyr-Pro-Phe-Phe-NH_2) were isolated from bovine brain in 1997. They have shown high selectivity toward μ-opioid receptor with potent analgesic activity[1,2]. The aromatic amino acids (Tyr^1, Phe^3, or Phe^4) of EM-2 are important in interacting with the opioid receptors besides the proline residue at the second position conferring high selectivity on the μ-opioid receptor[3]. Recent researches have reported that the exogenous application of these opioid peptides generally met with failure, owing to their biological instability and inability to be transmitted through the blood-brain barrier (BBB)[4]. In order to avoid these disadvantages and increase their affinity and selectivity, several analogs were synthesized and evaluated.

Fourier transform mass spectrometry (FT-MS) is an important tool in qualitative structural analysis of biomolecule. FT-MS has excelled in qualitative structural analysis due to its high mass resolving power, mass accuracy measurements, and multistage tandem mass spectrometry[5]. Electrospray ionization (ESI), especially when combined with tandem mass spectrometry, has played an increasingly important role in the analysis of peptide over the last two decades[6]. From the tandem MS fragmentation pattern of a peptide, its amino acid sequence can be deduced. Although, in principle, fragmentation of a peptide may yield six different sequence-specific series (a, b, and c type from N-terminus, and x, y, and z type from C-terminus), not all ions are formed with equal facility[7,8]. Even if one of these six ion series is complete or overlapping members of two partial sequence ion series are observed, then the sequence of a peptide can be deduced from its mass spectrum[8,9].

In this study, we investigated a series of C-terminal modified analogs of endomorphin-2 (compound 1—4 shown in Scheme 1) by ESI-FT-ICR-MS. The sequence

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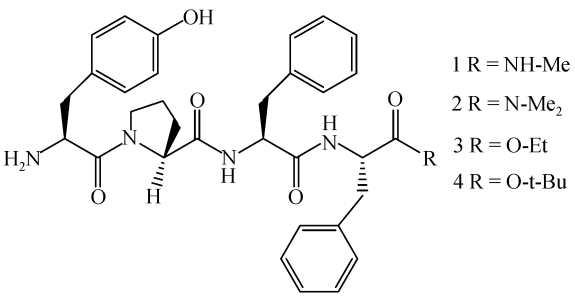
†Corresponding author (email: wangrui@lzu.edu.cn; yxcui@bjmu.edu.cn)

of peptide analogs and the modified groups were obtained.



Scheme 1 Structure of peptide analogs.

1 Experimental section

1.1 Materials

All of these peptide analogs were synthesized by standard liquid-phase synthesis. The detailed experimental procedures and biological activities will be reported in detail elsewhere. Acetic acid and methanol were bought from Beijing Chemical Factory (Beijing). Angiotensin II was used as standard from Sigma (St. Louis, MO).

1.2 ESI/CID MS

All positive-ion electrospray mass spectra were recorded using a Bruker APEX IV 7.0T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, MA USA) equipped with an external ESI source (Analytica, Branford Inc. USA). Peptide analogs were dissolved in a solvent system composed of methanol/water/acetic acid (V:V:V = 49:49:2), to give a concentration of approximately 0.3 μg/mL. Solutions were electrosprayed from a capillary (ID: 0.5 μm) biased at –4 kV. Typically, the infusion rate was 60 μL/h. The scan range was from *m*/*z* 100 to 700. During tandem MS experiments, the selected ions $[M+H]^+$ collided with argon gas, thus producing the fragment ions. External calibration was accomplished using Angiotensin II as standard.

2 Results and discussion

Benefiting from the high accurate measurement of the ESI-FT-ICR-MS, the sequence analysis of our peptide analogs was performed successfully. The MS and MS^2 spectra of compound 1 are shown in Figure 1, which are discussed as a typical example. The fragment ions of compound 1 listed in Table 1 are named according to the suggestion of Roepstroff and Fohlman[10]. Actually, we observed y" ion series instead of y, where y"=y+2.

Compound 1 gave the protonated molecule $[M+H]^+$ at *m*/*z* 586, and the Na^+ adduct ion $[M+Na]^+$ at *m*/*z* 608 as the main ion peaks in the positive ESI mass spectrum (Figure 1 up), respectively. Some backbone fragments, such as b_4, a_4, $y"_3$, and b_3, were also obtained with low intensity at *m*/*z* 555, 527, 423, and 408, respectively.

The MS^2 spectrum of compound 1 showed quasi-molecular ion $[M+H]^+$ at *m*/*z* 586. A small satellite peak at *m*/*z* 568 was most likely due to loss of H_2O from $[M+H]^+$. A complete sequence of Tyr-Pro-Phe-Phe-NH-CH_3 can be derived through the presence of various N- and C-termini sequence ions in this spectrum. For example, the ions at *m*/*z* 261, 408, and 555 were b_2, b_3, and b_4 N-terminus sequence ions. Ion b_1 was missing. This is likely due to the increased basicity of the backbone amide at the Tyr-Pro bond. The a_1, a_2, a_3, and a_4 ions were also present at *m*/*z* 136, 233, 380, and 527, respectively. The loss of NH_3 (17 u) from a_3 and a_4 ions were also detected. The spectrum also contained the y''_1, y''_2, and y''_3 ions at *m*/*z* 179, 326, and 423. Peptides with Pro often yield abundant internal frament ions from cleavage adjacent to Pro; therefore, several amino-acylium internal fragments were present at *m*/*z* 392 (PFF), 295(FF), and 245(PF). The corresponding amino-immonium internal fragments were also visible at masses 28 u lower than the mass of the amino-acylium ions except PFF. Two weak peaks observed at *m*/*z* 390 and 243 resulted from the loss of hydrogen from PFF and PF, respectively. The low mass region of CID spectra often contains valuable information in the form of ions generated from the individual amino acids present in the peptide. For compound 1, the presence of Phe and Tyr can be deduced from their immonium ions at *m*/*z* 120 and 136, respectively[11]. Since the scan range was from *m*/*z* 100, the immonium ion of Pro at *m*/*z* 70 could not be detected.

Therefore, the complete amino acid sequence of compound 1 was determined as Tyr-Pro-Phe-Phe and the C-terminal modified group was verified to be methyl-amino by the exact mass difference between $[M+H]^+$ and b_4.

The high mass resolution of FT-MS shows its powerful capability now. By subtracting the neighbored b or y″ series ions, we can read the amino acid from N-

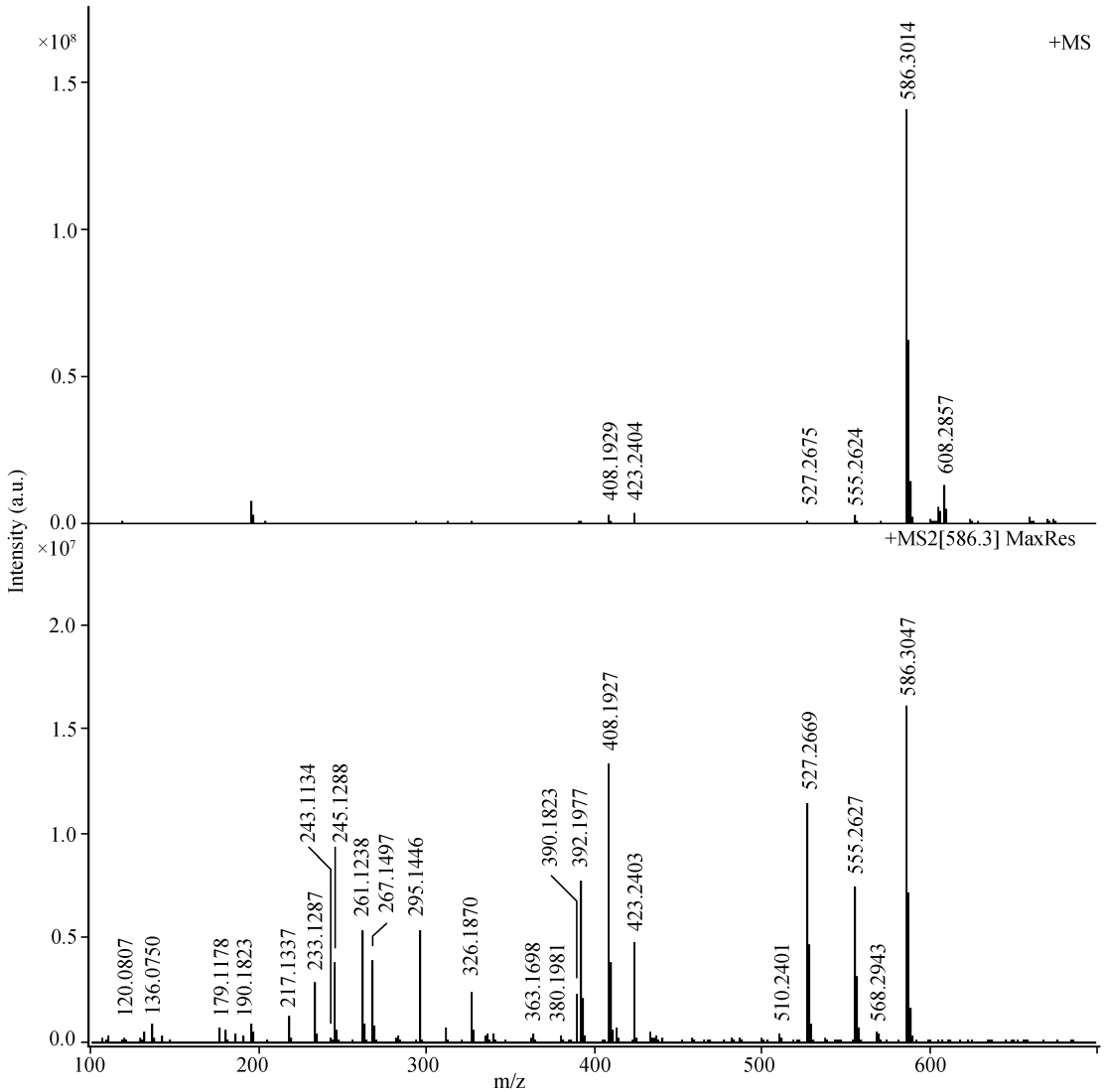


Figure 1 The MS (up) and MS^2 (down) spectra of compound 1.

or C-terminal directly. In all kinds of peptide sequencing methods, only FT-MS can make it. Furthermore, with ultra-high resolution, nearly all peaks can be assigned unambiguously. For example, there is little difference between the mass of y''_3 and c_3 ion. The former is 423.2391 while the latter is 423.2027. However, it is easy for FT-MS to differentiate them. Therefore, the peak observed at *m*/*z* 423.2403 must be assigned to y''_3 instead of c_3.

The other compounds, 2 and 3, possessed very similar mass spectral fragmentation patterns in ESI-FT-ICR-MS^2. However, comparing the MS^2 spectrum of compound 4 (Figure 2) with compound 1, there were apparent differences even if a, b, and internal ions of these two compounds were all the same. First, a high peak at *m*/*z* 573 was detected which did not belong to any backbone fragment. Second, the y″ series ions should have the masses of 222, 369, and 466, but none of them was observed, whereas another three peaks *m*/*z* at 166, 313, and 410 appeared in the spectrum. Interestingly, the mass differences between these two groups of data were exactly equal (detailed in Table 2). Based on these differences, we can determine the lost neutral fragment as butene whose mass is 56.0626. The only reasonable conclusion that can be drawn from these facts is that a McLafferty rearrangement occurred in C-terminal modified group which is shown in Scheme 2.

3 Conclusion

In this paper, a set of C-terminal modified analogs of endomorphin-2 was investigated by ESI-FT-ICR-MS^2.

Table 1 ESI FT-ICR MS^2 fragment ions of compound 1

Ion	Composition	*m*/*z* (cal)	*m*/*z* (exp)	Error ($\times 10^{-6}$)	Intensity (%)
M+H	$C_{33}H_{40}N_5O_5$	586.3024	586.3047	3.9	100.0
M+H–H_2O	$C_{33}H_{38}N_5O_4$	568.2918	568.2943	4.4	3.0
b_1	$C_9H_{10}N_1O_2$	164.0706	–	–	0
b_2	$C_{14}H_{17}N_2O_3$	261.1234	261.1238	1.5	33.8
b_3	$C_{23}H_{26}N_3O_4$	408.1918	408.1927	2.2	82.9
b_4	$C_{32}H_{35}N_4O_5$	555.2602	555.2627	4.5	46.1
y''_1	$C_{10}H_{15}N_2O_1$	179.1179	179.1178	–0.6	3.5
y''_2	$C_{19}H_{24}N_3O_2$	326.1863	326.1870	2.1	14.5
y''_3	$C_{24}H_{31}N_4O_3$	423.2391	423.2403	2.8	30.4
a_1	$C_8H_{10}N_1O_1$	136.0757	136.0750	–5.1	2.0
a_2	$C_{13}H_{17}N_2O_2$	233.1285	233.1287	0.9	18.0
a_3	$C_{22}H_{26}N_3O_3$	380.1969	380.1981	3.2	1.6
a_3—NH_3	$C_{22}H_{23}N_2O_3$	363.1703	363.1698	–1.4	2.5
a_4	$C_{31}H_{35}N_4O_4$	527.2653	527.2669	3.0	71.8
a_4—NH_3	$C_{31}H_{32}N_3O_4$	510.2387	510.2401	2.7	2.2
PFF	$C_{23}H_{26}N_3O_3$	392.1969	392.1977	2.0	47.8
PFF—2H	$C_{23}H_{24}N_3O_3$	390.1812	390.1823	2.8	14.0
FF	$C_{18}H_{19}N_2O_2$	295.1441	295.1446	1.7	33.5
FF—CO	$C_{17}H_{19}N_2O_1$	267.1492	267.1497	1.9	24.0
PF	$C_{14}H_{17}N_2O_2$	245.1285	245.1288	1.2	24.1
PF—CO	$C_{13}H_{17}N_2O_1$	217.1335	217.1337	0.9	7.9
PF—2H	$C_{14}H_{15}N_2O_2$	243.1128	243.1134	1.2	0.4
F—CO	$C_8H_{10}N_1$	120.0808	120.0807	–0.8	0.5

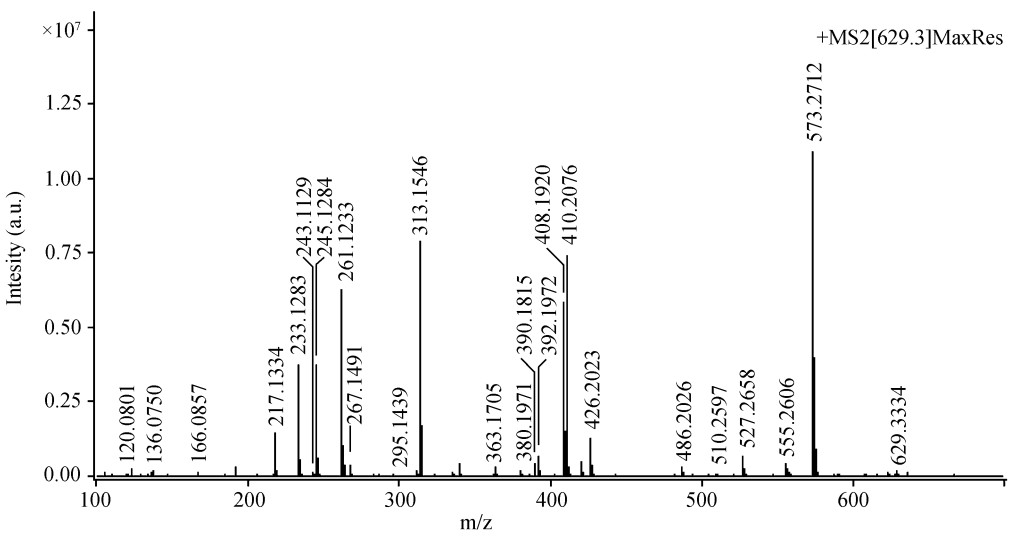
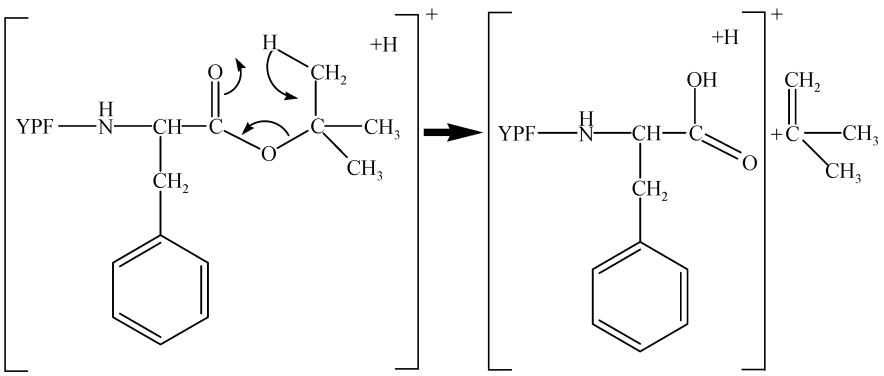


Figure 2 The MS^2 spectrum of compound 4.

Table 2 The mass differences between calculated quasi-molecule ion, y" series ions, and observed unknown ions

calculated $[M+H]^+$ and y″ series ions (*m*/*z*)	observed unknown ion (*m*/*z*)	mass difference
629.3334[a)]	573.2712	56.0622
222.1489[b)]	166.0857	56.0632
369.2173[c)]	313.1546	56.0627
466.2700[d)]	410.2076	56.0624

a) Mass of calculated $[M+H]^+$; b) mass of calculated y''_1; c) mass of calculated y''_2; d) mass of calculated y''_3.



Scheme 2 McLafferty rearrangement of compound 4.

From the good match of the mass between the calculated a, b, y", and internal ions and the observed ones, we determined the sequence and modified groups of these peptide analogs. Moreover, if the C-terminal modified group is t-butyloxy, it can lose butene through McLafferty rearrangement in MS^2 experiment. The ESI-FT-ICR-MS CID is proved to be a powerful tool for biomolecule analysis in this study. This provides a rapid, convenient, and general method for peptide sequence determination.

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