

sequence-scrambled fragment ions from

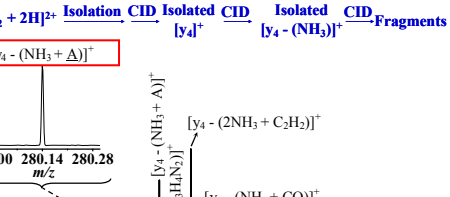
scrambled fragment ions from y-type ions are

presence of sequence scrambling [1] in concrete evidence has been provided on fragment ions (e.g., c, x, y, and z).

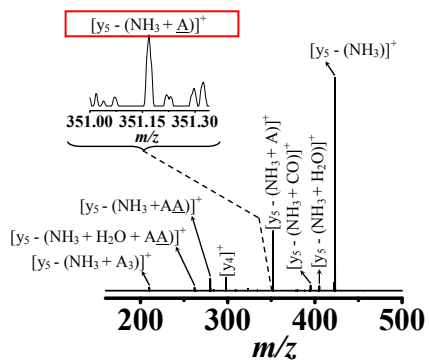
or absence (and extent) of sequence-scrambled collision-induced dissociation (CID) type ions will be discussed to determine sequence-scrambled fragment ions; we demonstrate that sequence-scrambled fragment ions similar to “b”- and “a”-type fragments are observed.

melanocyte, methanol, and water were used. Melanin II was purchased from AnaSpec Inc. Peptides were purchased from CPC Scientific (CPC Scientific) of the abovementioned peptides in duplicate and separately and for the electrospray

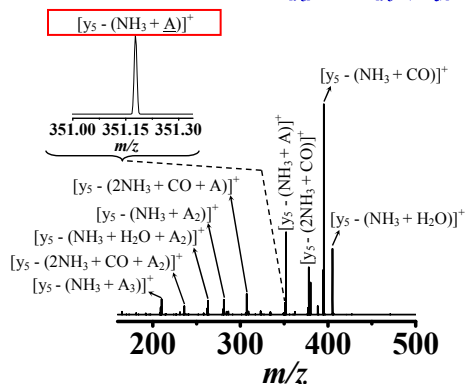
using a Thermo LTQ/Orbitrap mass spectrometer operated in positive ion-mode ESI. The voltages were set at -3 kV, +50 V, and +105 V, respectively. The ions were formed in the LTQ. Optimized collision energy (normalized collision energy) in the range of 15 % to 30 % was used for the CID experiments, helium (He) was used as the collision gas, and the activation “q” parameter of the ion trap was set to 0.25. The CID fragment ion assignments were < 4 eV. The identities should not be confused with other fragment ions.



(a) [AAAAHAA-NH<sub>2</sub> + 2H]<sup>2+</sup> Isolation CID Isolated CID-Fragments

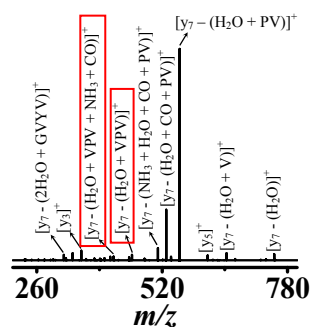


(b) [AAAAHAA-NH<sub>2</sub> + 2H]<sup>2+</sup> Isolation CID Isolated CID-Fragments

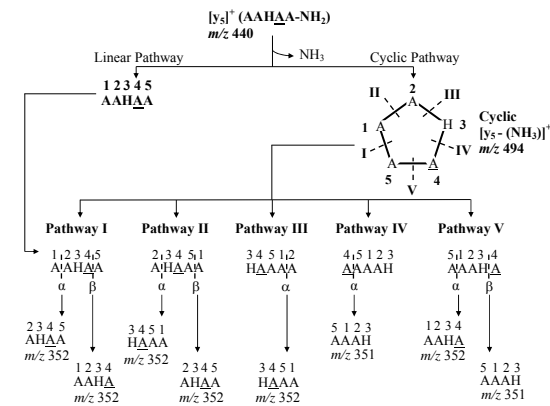
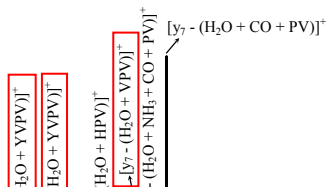


## (II) CID of $y_7^+$ from Angiotensin II (EGVYVHPV)

(a) In-source  $[y_7]^+$  (GVVYVHPV) Isolation CID-Fragments

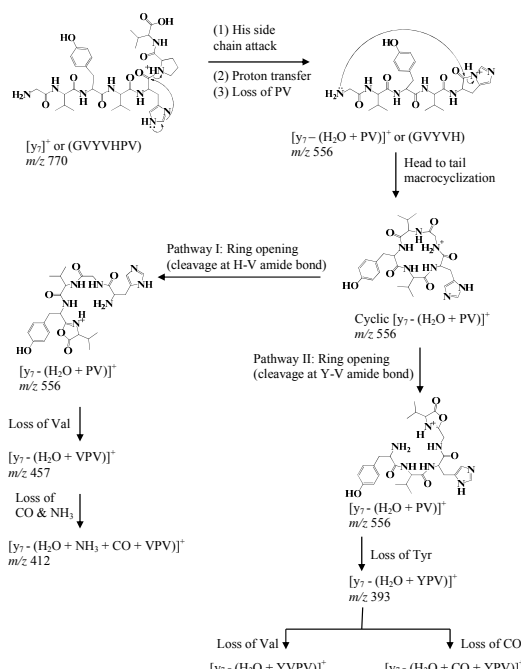


(b) In-source  $[y_7]^+$  (GVVYVHPV) Isolation CID Isolated CID-Fragments



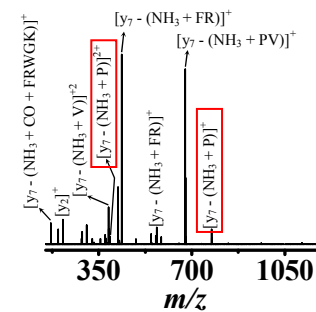
**Scheme 1.** Possible pathways for formation of fragment ions at  $m/z$  351 and 352 generated from CID of  $[y_5]^+$  (from AAAHAA-NH<sub>2</sub>).

**Figure 2.** Collision-induced dissociation (CID) mass spectra of  $m/z$ -isolated (a)  $[y_5]^+$  ( $m/z$  440) (generated from CID of doubly-charged AAAAHAA-NH<sub>2</sub>) and (b)  $[y_5 - (NH_3)]^+$  ( $m/z$  423) (generated from CID of  $[y_6]^+$ ) at 20 % normalized collision energy. Insets in panels (a) and (b) show the expanded views of  $m/z$  range of 351.0 to 351.3 corresponding to identified sequence-scrambled fragment ion at  $m/z$  351 (i.e.,  $[y_5 - (NH_3 + \Delta)]^+$ ). The scrambled fragment ions are shown in red boxes.

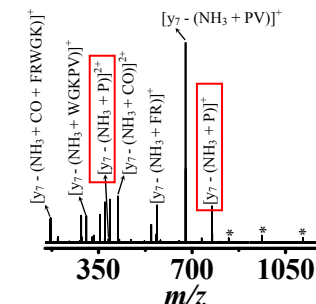


## (II) CID of $y_7^{2+}$ from Des-Acetylated- $\alpha$ -Melanocyte

(a) [SYSMEHFRWGKPV-NH<sub>2</sub> + 3H]<sup>3+</sup> Isolation CID Isolated CID-Fragments



(b) [SYSMEHFRWGKPV-NH<sub>2</sub> + 3H]<sup>3+</sup> Isolation CID Isolated CID-Fragments



**Figure 4.** Collision induced dissociation (CID) mass spectra of  $m/z$ -isolated  $[M + 3H]^{3+}$  of des-acetylated- $\alpha$ -melanocyte and (b)  $[y_7 - (NH_3)]^{2+}$  (generated from CID of  $[y_8]^{2+}$ ) at 20 % normalized collision energy. Sequence-scrambled fragment ions are shown in red boxes. Peaks corresponding to electronic noise are marked with asterisks.

**Table 1.** Summary of the total number of observed sequence-scrambled fragment ions generated from CID of y fragment ions.

$y_n$ Fragment Ion	Charge State	Total Frag.
Des-acetylated- $\alpha$ -melanocyte		
$y_6$ (GKPV)	1	1
$y_6$ (WGKPV)	1	1
$y_6$ (RWGKPV)	1	1
$y_6$ (FRWGKPV)	1	1
$y_6$ (RWGKPV)	2	2
$y_7$ (FRWGKPV)	2	2
$y_6$ (EHRWGKPV)	2	2
$y_6$ (MEHRWGKPV)	2	2
$y_7$ (SMEHRWGKPV)	2	2
$y_6$ (BHRWGKPV)	3	3
$y_6$ (MEHRWGKPV)	3	3
$y_6$ (YSMEHRWGKPV)	3	3
Angiotensin II		
$y_7$ (HPV)	1	1
$y_7$ (VHPV)	1	1
$y_7$ (GVVYVHPV)	1	1
$y_7$ (GVVYVHPV)	2	2
$y_7$ (GVVYVHPV)	2	2

## CONCLUSIONS

- CID results presented here provide evidence for the presence of sequence-scrambled fragment ions.
- We examined the sequence-scrambled collision-induced dissociation (CID) product ions of  $[y_n]^{m+}$  ( $n = 3$  to  $12$ ,  $m = 1$  to  $3$ ) from various peptides.