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Gas-phase fragmentation of deprotonated tryptophan and its clusters $[\mathrm{Trp}_n{-}\mathrm{H}]^-$ induced by different activation methods

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Keywords:	: singly deprotonated tryptophan clusters, tandem mass spectrometry, collision-induced dissociation, electron-induced dissociation
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Gas-phase fragmentation of deprotonated tryptophan and its clusters $[Trp_n{-}H]^-$ induced by different activation methods[§]

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[§] Dedicated to the memory of the late Professor Nico M. M. Nibbering.

Abstract

Fragmentation reactions of deprotonated tryptophan (Trp), [Trp−H]$^-$ and Trp singly deprotonated non-covalently bound clusters [Trp$_n$−H]$^-$, where n = 2, 3, 4, were investigated using low-energy collision-induced dissociation (CID) with He atoms, high-energy CID with Na atoms, and electron-induced dissociation (EID) with 20 – 35 eV electrons. Fragmentation of monomeric Trp anion, where all labile hydrogens were exchanged for deuterium [d_4-Trp−D]$^-$, was investigated using low-energy CID and EID, in order to shed light on the dissociation mechanisms. However, the anion undergoes scrambling prior to the dissociation, likely in the form of ion-molecule complex. The main fragmentation channel for Trp cluster anions, [Trp$_n$−H]$^-$, n > 1, is the loss of the neutral monomer. The fragmentation of deprotonated Trp monomer induced by electrons resembles the fragmentation induced by high-energy collisions through electronic excitation of the parent. However, the excitation must precede in a different way, shown through only monomer loss from larger clusters, n > 1, in case of EID, but intracluster chemistry in the case of high-energy CID.

Keywords: singly deprotonated tryptophan clusters; tandem mass spectrometry; collision-induced dissociation; electron-induced dissociation;

Running Title: Fragmentation of [Trp$_n$−H]$^-$.

Introduction

Tryptophan (Trp) is an essential amino acid, crucial for biological functioning of proteins and enzymes.[1] It is a metabolic precursor of vitamin B5 (niacin) and of the hormone and neurotransmitter 5-hydroxytryptamine (serotonine).[2 and refs therein]

Fragmentation of neutral Trp[3-6] and protonated Trp[7-12], i.e. $[Trp+H]^+$, has been subject of several studies using various fragmentation methods. Fragmentation of the Trp radical cation $Trp^{\bullet +}$ showed different dissociation mechanism to even-electron protonated Trp $[Trp+H]^+$.[13] Photodetachment of deprotonated Trp, *i.e.* $[Trp{-}H]^-$ showed sensitivity of the Trp chromophore to the presence of remote negative charge.[14] Fragmentation of deprotonated amino acid anions including Trp was investigated by Bowie and co-workers[15-17] and in a review[15] Bowie outlined four major classes of fragmentation processes of organic negative ions including amino acids. These proceed through: (i) homolytic cleavage where loss of a radical forms stable radical anion; (ii) formation of anion complex; (iii) proton transfer toward deprotonated site thus forming a new anion; and (iv) rearrangement reactions. Positive- and negative-ion fragmentation spectra can be very different and thus provide useful complementary structural information for peptides.[18-21] Additionally, the negative-ion fragmentation reactions of peptides are useful in identification of post-translational modifications[18,22-24] as well as more complete series of backbone fragments in peptide sequencing.[20,25]

Non-covalent amino acid clusters have been subject of intense research over past 20 years and covered diverse areas of interest, e.g. the use of fragmentation of proton-bound dimers to determine the gas-phase proton affinities[26] or methylating abilities,[27] peptide bond formation within amino acid clusters,[28] formation and ionisation of neutral non-covalent amino acid complexes,[29-31] or formation and fragmentation reactions of multiply charged clusters using various activation methods.[32-38] Most of the research has focused on positive ions and only few report on anionic clusters of amino acids.[33,38]

Thus, in the present work we explore the fragmentation pattern of deprotonated Trp $[\mathrm{Trp{-}H}]^-$ after high-energy collisions with Na atoms, low-energy collisions with He and 25 - 30 eV free electrons. Mass spectra resulting from the experiments using collision-induced dissociation (CID) and electron-induced dissociation (EID) are presented. In addition, we have examined the unimolecular fragmentation chemistry of deprotonated Trp clusters $[\mathrm{Trp}_n{-}\mathrm{H}]^-$, where $n = 2, 3, 4$, with these different activation methods. The use of collisions with sodium atoms offered an opportunity to look for the possibility of collisional electron transfer to the clusters to form doubly charged anions.[39]

Experimental

L-Tryptophan (purity ≥ 98%) was used as received from Sigma-Aldrich (Milwaukee, USA). All experiments were carried out using a 1 mM solution of tryptophan in MeOH on two different instruments: (i) a Finnigan- LTQ-FT (Thermo, Bremen, Germany) mass spectrometer equipped with electrospray ionization (ESI) source[40] described in detail elsewhere;[41] (ii) Separator Experiment described in detail previously.[42] For labelling studies, Trp was dissolved in MeOD, which resulted in exchanging the labile H for D, thus exchanging four hydrogens in the neutral Trp.

(i) Low-energy CID and EID spectra on the LTQ-FT

The tryptophan solution was introduced to the mass spectrometer at 5.0 µL/min via ESI in the negative mode. Typical ESI conditions used were: spray voltage, 2.2 – 4.0 kV, capillary temperature, 200 – 250 °C, nitrogen sheath pressure, 5 – 25 (arbitrary units). The capillary voltage and the tube lens offset were tuned to maximize the desired peak. The injection time was set using the automatic gain control function. The LTQ-FT mass spectrometer consists of: (i) linear ion trap (LTQ); (ii) ion transfer optics; and (iii) FT-ICR mass analyzer. For the tandem mass spectrometry experiments, the desired ions produced via ESI were mass selected, trapped in the LTQ and subjected to CID at a He bath gas pressure of ca. 5×10^{-3} Torr at the room temperature. CID was carried out by mass selecting the desired ions with a 1.5 – 5 *m/z* units window and subjecting them to the following typical conditions: normalized collision energy between 16 and 40, which determines the translational kinetic energy of the ions; activation (Q) 0.25 – 0.35, which assigns the RF frequency used to fragment ions; and activation time of 30 ms that is the time set to excite the ions via CID. For high resolution mass analysis and EID, the ions were transferred via the ion optics transfer region ($\sim 2 \times 10^{-7}$ Torr) into an FT-ICR cell at a pressure below 1.5×10^{-9} Torr. The FT-ICR cell is supplied with low-energy electrons produced by an indirectly heated emitter cathode located downstream of the FT-ICR cell. The energy of electrons is given by the potential difference between the emitter cathode with *ECD offset* of −3.2 V and the grid positioned in front of the cathode, which is variable. Ions were bombarded with electrons of energies 20 - 35 eV for 30 – 100 ms. These conditions were carefully selected to maximise the fragment ion signal intensity and minimize secondary electron ion interaction in the cell.[9,37]

(ii) High-energy CID on a sector instrument

The tryptophan solution was introduced to the mass spectrometer via ESI in the negative mode. Ions were accelerated to 50 keV, *m/z* selected by a bending magnet and passed through two collision chambers before the product ions were analysed by a hemispherical electrostatic analyser.[43,44] A heated cell containing Na allowed for collisions with Na atoms in the first collision chamber. The second collision chamber was not used in the present experiments.

Results and Discussion

ESI of a 1 mM solution of tryptophan (solvent: MeOH) yields a series of cluster ions of the type $[\mathrm{Trp}_n\mathrm{-xH}]^{x-}$, where $x = 1 - 4$, as observed in the recent study.[38] However, the present work focuses on singly charged tryptophan cluster, i.e. $x = 1$, $[\mathrm{Trp}_n\mathrm{-H}]^-$, $n = 1, 2, 3, 4$.

Fragmentation of [Trp−H]$^-$ and [d$_4$-Trp−D]$^-$

Figure 1 shows low-energy CID in (a), high-energy CID in (b), and EID of $[\mathrm{Trp-H}]^-$ in (c). The mass selected precursor ion is always denoted by a star. Low-energy CID performed in the linear ion trap is slow heating process, resulting in fragmentation of ions through vibrational excitation. The low-energy CID of deprotonated tryptophan $[\mathrm{Trp-H}]^-$ shows a number of fragment ions (Figure 1a). The dissociation pathways for $[\mathrm{Trp-H}]^-$ are summarised in Scheme 1, eqs. 1 - 7, and the fragment ions resulting from the dissociation of $[\mathrm{Trp-H}]^-$ are shown in Scheme 2. The $[\mathrm{Trp-H}]^-$ is likely to be formed as the carboxylate anion, as the carboxylic acid group is the most acidic site[45] and ESI generated $[\mathrm{Trp-H}]^-$ has been shown to exist as the carboxylate via the use of gas-phase IR spectroscopy.[46] Indeed, the most abundant fragment ion at *m/z* 159 arises via decarboxylation of $[\mathrm{Trp-H}]^-$ (eq. 1). Decarboxylation of $\mathrm{RCH_2CO_2}^-$ has been reported and observed if the electron affinity of $\mathrm{R}^\bullet$ is positive.[15,17] Bowie suggested that [15] the loss of NH_3 proceeds through intramolecular H^+ abstraction from $C_\beta H_2$ by carboxylate, and formation of an ion-molecule complex that leads to the formation of fragment ion at *m/z* 186 after dissociation (eq. 2). This fragment ion was also observed in the fast atom bombardment-collisional activation (FAB-CA) of $[\mathrm{Trp-H}]^-$.[16]

In order to shed light onto this process, Trp, where all labile hydrogens were exchanged for deuterium, was investigated by low-energy CID, see Figure 2a. One can see that $[\text{Trp–D}]^-$ undergoes complete intramolecular scrambling prior dissociation. This was observed earlier in the positive case $[\text{Trp+D}]^+$.[7,47] The presence of the fragment at *m/z* 187 due to the loss of NHD_2 supports the suggestion that the H has been abstracted from C_β. However, the observation of fragment at *m/z* 186, which is due to the loss of ND_3, suggests abstraction from indole ring. Loss of NH_2D forming fragment ion at *m/z* 188 suggests one of the D on the initial ND_2 group underwent H/D exchange in an ion-molecule complex prior to dissociation. Similarly, as suggested earlier by Bowie,[15] the CO_2 + NH_3 are lost through the same ion-molecule complex forming fragment at *m/z* 142 (eq. 3). The multistage mass spectrometry experiments have revealed that the CO_2 is lost from the fragment ion at *m/z* 186 that has lost NH_3 initially (data not shown). The ion abundances of fragment ions corresponding to loss of NH_3 and CO_2 in deuterated Trp (Figure 2a), i.e. fragment ions at *m/z* 186 (loss of ND_3), *m/z* 187 (loss of NHD_2), and *m/z* 188 (loss of NH_2D), resemble the relative ion abundances of *m/z* 142 (loss of CO_2 + ND_3), *m/z* 143 (loss of CO_2 + NHD_2), and *m/z* 144 (loss of CO_2 + NH_2D), respectively. The second most abundant fragment ion in the low-energy dissociation of $[\text{Trp–H}]^-$ (Figure 1a) is the ion at *m/z* 116, which is negatively charged indole ring (eq. 6). It is an unusual decomposition as noted by Bowie and co-workers,[16] but it is present in all spectra in Figure 1 with relatively high abundance. Figure 2a shows H – D scrambling for the corresponding peaks at *m/z* 117 – 119, where the most abundant is the indole ring with two deuteriums. Possibly the H has been abstracted from C_α (eq. 6) forming ion-molecule complex that underwent H – D exchange before dissociation. The fragment ion at *m/z* 116 showed to come also from further dissociation of the fragment ion at *m/z* 159 (Figure 1a), but in a low abundance. The fragment ion at *m/z* 130 can likely proceed through abstraction of H from NH_2 group forming ion-molecule complex that can undergo further scrambling as shown in the deuterated case in Figure 2a, eq. 4. MS^3 experiments shown that this fragment comes also from the fragment ion at m/z 159. High resolution mass spectrometry confirmed that the fragment ion at m/z 173 is formed through loss of the amino methyl radical. $\bullet CH_2NH_2$, which likely forms through an ion-molecule complex (eq. 7). Loss of such a radical was observed in the case of deprotonated glycine,[17] but has not been observed via high-energy CID (Figure 1b), FAB-CA,[16] or EID (Figure 1c). Minor loss of the side chain forms the fragment ion $H_2N^-CHCO_2H$ at m/z 74, through intramolecular abstraction of H^+ from NH_2 by the carboxylate to form an ion-molecule complex prior dissociation (eq. 5) that undergoes scrambling as shown in deuterated case (Figure 2a).

High-energy CID and EID leads to similar fragmentation spectra with relatively similar abundances of the formed fragment ions. There was no sign of the formation of dianions in collisional electron transfer from sodium. The most abundant fragment ion observed in the high-energy CID spectrum is the ion at m/z 158 (Figure 1b), due to the loss of $\bullet CO_2H$, with a smaller amount of a fragment ion forming at m/z 159 due to the loss of CO_2, which has been also observed in the low-energy CID. In the EID spectra, both ions are of similar abundance (Figure 1c). The EID of deuterated Trp anion (Figure 2b) resulted in the loss of $\bullet CO_2H$ with a slightly higher abundance than the loss of CO_2. A minor loss of $\bullet CO_2D$ was also observed, suggesting the H could be lost from two different positions of the Trp anion. In the EID spectra of $[Trp-H]^-$ a loss of H• is observed. Bowie suggested the loss of H• to come from C_α carbon.[15] This is supported by observation of loss of H• in the EID of $[d_4\text{-Trp}-D]^-$ (Figure 2b)), but loss from C_β cannot be excluded. However, in the case of EID loss of H• from $[Trp+Na]^+$, the loss from C_α is preferred, supported by the DFT calculations.[47] Fragment ion at m/z 142 due to the loss of CO_2 and NH_3 is observed in the high-energy CID and EID as in the case of low-energy CID. However, this fragment ion is missing in the EID spectra of deuterated Trp anion $[d_4\text{-Trp}-D]^-$ (Figure 2b). As already mentioned, the formation of an indole ring negative fragment ion at *m/z* 116 is observed in all the activation methods. In the case of EID of the deuterated Trp anion (Figure 2b), the fragment anions of the indole ring are observed, possessing one D at *m/z* 117 and two D at *m/z* 118. Thus, some scrambling must have taken place prior the dissociation. Loss of a side chain forming negatively charged backbone at *m/z* 74 (eq. 5) is observed with the highest abundance in the high-energy CID. High-energy CID and EID closely resembles the fragmentation spectra likely due to the similarity of the activation method leading to fragmentation through electronic excitation of $[Trp{-}H]^-$.

[Insert Figure 1 and 2 here, please]

Fragmentation of $[Trp_2{-}H]^-$

Dissociation spectra for deprotonated dimer $[Trp_2{-}H]^-$ are shown in Figure 3 for low-energy CID (a), high-energy CID (b) and EID (c). Vibrational excitation of the dimer leads to the neutral Trp loss and further loss of CO_2 from the monomer $[Trp{-}H]^-$. No intracluster chemistry is observed as was in the case of some proton bound dimers.[27,41,48] The loss of monomer is dominant channel in all three activation methods studied. The high-energy CID and EID show differences in the fragmentation of the dimer $[Trp_2{-}H]^-$. While high-energy CID leads to several fragments of *m/z* above the monomer, EID shows preference for loss of a neutral Trp. This difference demonstrates the difference in the excitation induced by an electron and by high-energy collision with Na atoms for the deprotonated dimer $[Trp_2{-}H]^-$. It is noteworthy, that after high-energy collision, the constituents of the dimer are still non-covalently bound. High-energy CID leads to loss of 45, likely due to the loss of $\bullet CO_2H$, as we observed in the case of the monomer. Fragment ion at *m/z* 332 corresponds to extra mass of 129 amu to the mass of the monomer, which is likely the side chain of the Trp, thus the backbone of one of the Trp within the dimer has been lost. If the side chain is lost and the backbone remains bounded to the neutral Trp, this gives rise to the fragment ion at *m/z* 276 that is also observed, but with minor abundance. The fragment ion at *m/z* 249 is likely Trp bound to $HO^{-}C{=}O$.[15] Different relative abundance of the fragments of *m/z* 159, 158 and 116 in the EID of $[Trp_2{-}H]^{-}$, in comparison to the monomer $[Trp{-}H]^{-}$, suggest fragments at *m/z* 159 and 116 come from vibrational excitation of the Trp constituent within the cluster, as it resembles the relative abundance of these fragments in CID of the monomer (Figure 1a). However, the appearance of the fragment ion at *m/z* 158 must come from electronic excitation of the cluster. These fragment ions arise from fragmentation of the dimer, likely due to the concomitant loss of the monomer, i.e. the neutral Trp. Again there was no sign of collisional electron transfer from Na.

[Insert Figure 3 here, please]

Fragmentation of $[Trp_3{-}H]^{-}$

Figure 4 shows low-energy CID (a), high-energy CID (b) and EID (c) of the deprotonated Trp trimer $[Trp_3{-}H]^{-}$. Dissociation of the Trp within the cluster is observed only in the case of high-energy CID (Figure 4b). A dimer with an additional fragment corresponding to 45 amu is observed at *m/z* 452. The dominant fragmentation channel and the only one in the case of low-energy CID and EID is the loss of a neutral Trp (eq. 8).

[Insert Figure 4 here, please]

$$[Trp_n{-}H]^{-} \quad \rightarrow \quad [Trp_{n-m}{-}H]^{-} \; + \; m\,Trp \qquad (8) \qquad n > 1$$

Fragmentation of [Trp_4–H]$^−$

Figure 5 shows the low-energy CID (a) and high-energy CID (b) of the deprotonated Trp tetramer [Trp_4–H]$^−$. The observation of fragment ions at *m/z* values above that of the mass selected precursor anion, highlights that the tetramer is contaminated by the doubly charged octamer [Trp_8–2H]$^{2−}$. Doubly charged Trp clusters [Trp_n–2H]$^{2−}$ have been investigated recently in a detailed study of [Trp_9–2H]$^{2−}$ fragmentation induced by low-energy CID, ultraviolet photo-dissociation (UVPD) and EID.[38] The doubly charged fragment cluster ion [Trp_7–2H]$^{2−}$ observed through loss of a neutral Trp from [Trp_8–2H]$^{2−}$, was the smallest doubly charged cluster observed in the previous study.[38] Low-energy CID (Figure 5a) leads to competitive dissociation channels through loss of a neutral Trp from singly charged [Trp_4–H]$^−$ (eq. 8) and a charge explosion reaction of the doubly charged contaminant cluster [Trp_8–2H]$^{2−}$. In fact, recent high-resolution data[38] revealed that the signal from the apparent [Trp_4–H]$^−$ is largely due to the [Trp_8–2H]$^{2−}$. High-energy CID (Figure 5b) shows additionally loss of an electron forming radical cluster ion [Trp_8–2H]$^{•−}$. Loss of an electron was observed in the UVPD of doubly charged Trp cluster [Trp_9–2H]$^{2−}$.[38] Other fragments formed are similar to those observed in the low-energy CID due to the loss of neutral Trp molecules from [Trp_4–H]$^−$ and [Trp_8–2H]$^{2−}$ leading to fragment ions [Trp_n–H]$^−$, $n < 4$, and [Trp_7–2H]$^{2−}$, respectively. In the case of doubly charged parent ion [Trp_8–2H]$^{2−}$, neutral loss competes with charge separation of the cluster leading to singly charged Trp fragment clusters [Trp_n–H]$^−$ + [Trp_m–H]$^−$, where $n + m = 8$.

[Insert Figure 5 here, please]

Conclusions

Gas-phase fragmentation of deprotonated tryptophan and its non-covalently bound singly deprotonated clusters $[Trp_n{-}H]^-$, where $n = 1 - 4$, was investigated using low-energy CID with He atoms, high-energy CID with Na atoms, and free electrons of 20 – 35 eV in the EID. For $n = 1$, the main fragmentation channel in the low-energy CID is the loss of CO_2, whereas in the high-energy CID and EID the loss of $\bullet CO_2H$ competes with the loss of CO_2. The second most abundant fragment ion present in the spectra of all activation methods is the formation of the anion of indole ring $C_8H_6N^-$ at m/z 116. Noteworthy, a radical cleavage was observed in the low-energy CID, where Trp parent anion has lost $\bullet CH_2NH_2$. For $n > 1$, the main fragmentation channel is loss of neutral Trp. Only in the case of high-energy CID of clusters, minor fragments were observed, corresponding to the bond cleavages within the cluster. Thus, high-energy CID activates the parent cluster anion in a different way than EID. However, for $n = 1$, the high-energy CID and EID showed similar abundance of formed fragment anions. No evidence for the formation of dianions in collisional electron transfer from sodium was found. On a final note, there has been interest in the formation of peptide bonds within cluster ions as a possible route to prebiotic peptides.[28b] We find no evidence for this in the singly deprotonated clusters $[Trp_n{-}H]^-$.

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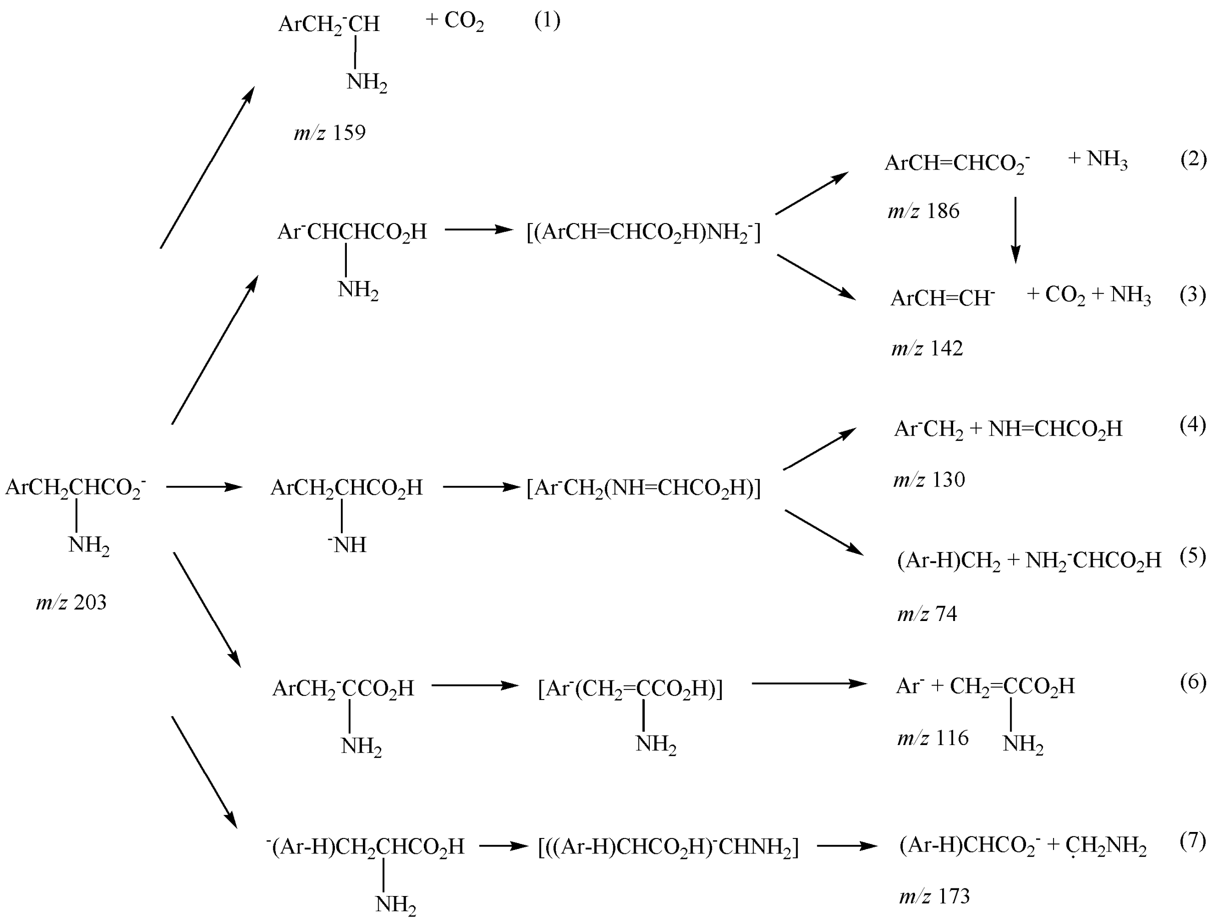
Scheme 1

Dissociation pathways for [Trp–H]$^-$. "Ar" stands for aromatic side chain of the Trp.



Scheme 2

Fragment ions resulting from the dissociation of [Trp–H]$^-$.

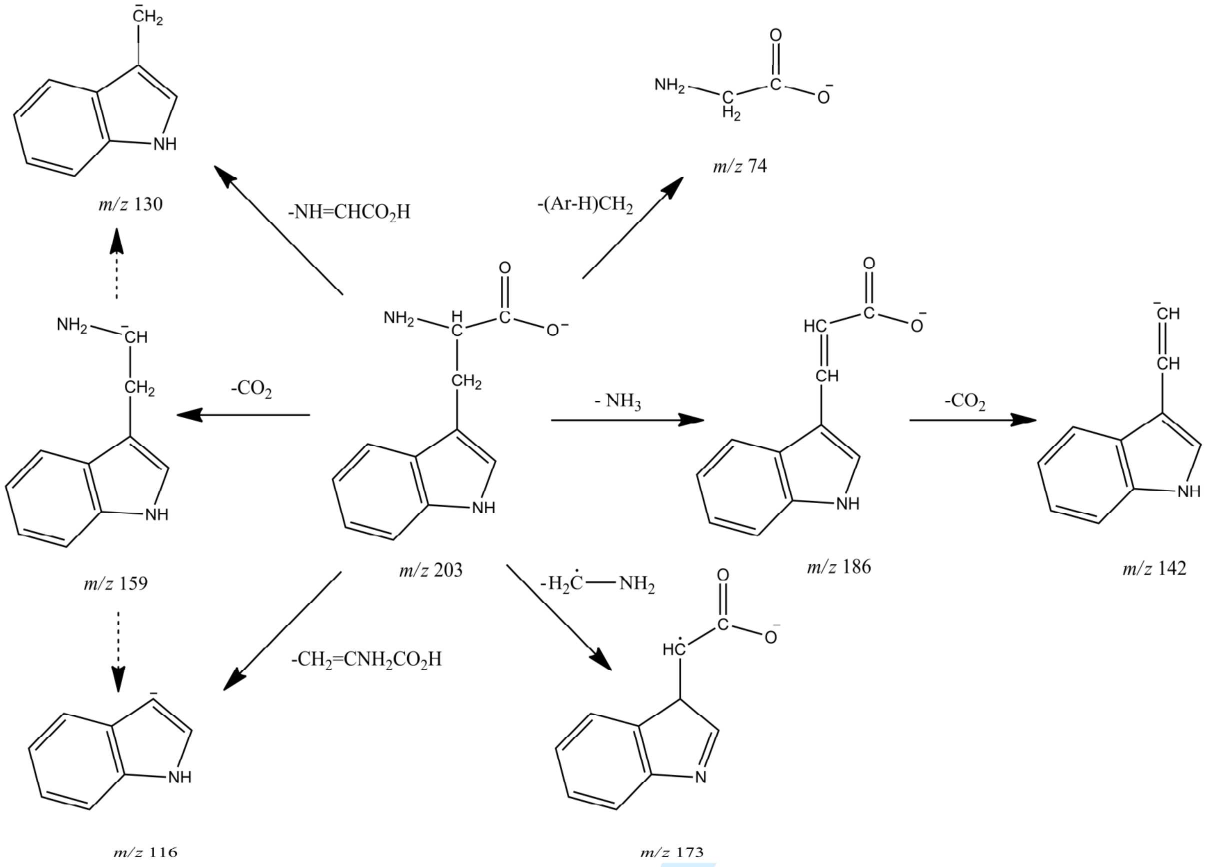


Figure captions

Figure 1: Fragmentation spectra of the monomer [Trp–H]$^-$ *m/z* 203: (a) low-energy CID in the linear ion trap (collision energy 34, activation time 30 ms); (b) high-energy CID with Na; (c) EID in the FT-ICR (electron energy 31.8 eV, activation time 30 ms).

Figure 2: Fragmentation spectrum of the deuterated monomer [d_4-Trp–D]$^-$ *m/z* 206: (a) at low-energy CID in the linear ion trap (collision energy 29, activation time 30 ms). Only the most abundant peaks are labelled for clarity. Adjacent peaks of Δ *m/z* = ± 1 correspond to same fragment with one more or less deuterium exchanged. All the peaks were confirmed using high-resolution FT-ICR mass analysis. (b) EID in the FT-ICR (electron energy 21.8 eV, activation time 70 ms.

Figure 3: Fragmentation spectra of the dimer [Trp$_2$–H]$^-$ *m/z* 407: (a) low-energy CID in the linear ion trap (collision energy 17, activation time 30 ms); (b) high-energy CID with Na; (c) EID in the FT-ICR (electron energy 27.4 eV, activation time 100 ms).

Figure 4: Fragmentation spectra of the trimer [Trp$_3$–H]$^-$ *m/z* 611: (a) low-energy CID in the linear ion trap (collision energy 19, activation time 30 ms); (b) high-energy CID with Na; (c) EID in the FT-ICR (electron energy 27.4 eV, activation time 40 ms).

Figure 5: Fragmentation spectra of the tetramer [Trp$_4$–H]$^-$ *m/z* 815: (a) low-energy CID in the linear ion trap (collision energy 20, activation time 30 ms); (b) high-energy CID with Na.

Figure 1:

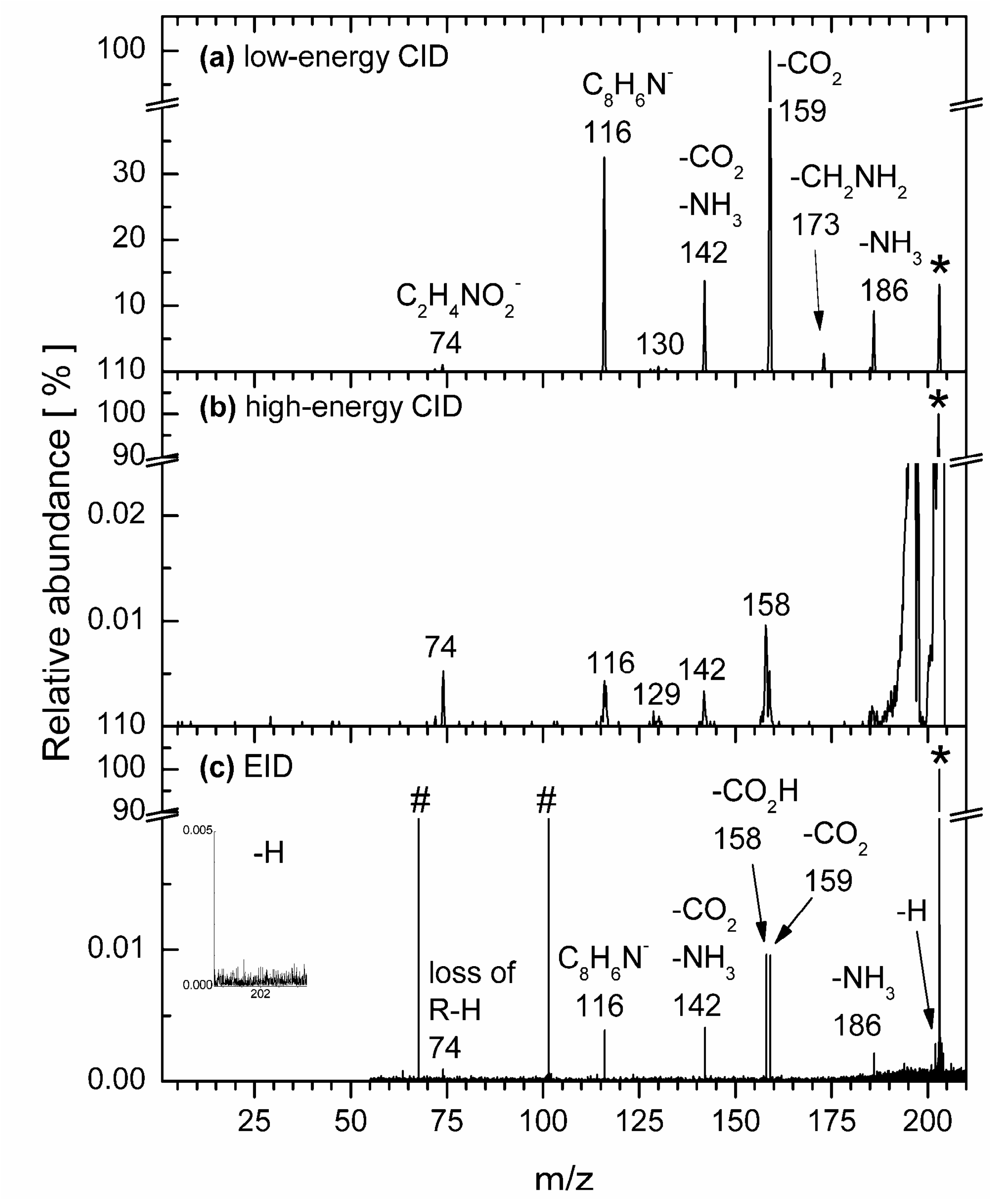

(a) low-energy CID
100
30
20
10
110
$C_2H_4NO_2^-$
74
$C_8H_6N^-$
116
130
$-CO_2$
$-NH_3$
142
$-CO_2$
159
$-CH_2NH_2$
173
$-NH_3$
186
*
(b) high-energy CID
100
90
0.02
0.01
110
74
116
129
142
158
*
(c) EID
100
90
0.01
0.00
0.005
0.000
202
-H
#
#
loss of
R-H
74
$C_8H_6N^-$
116
$-CO_2$
$-NH_3$
142
$-CO_2H$
158
$-CO_2$
159
$-NH_3$
186
-H
*
Relative abundance [%]
25
50
75
100
125
150
175
200
m/z

Figure 2:

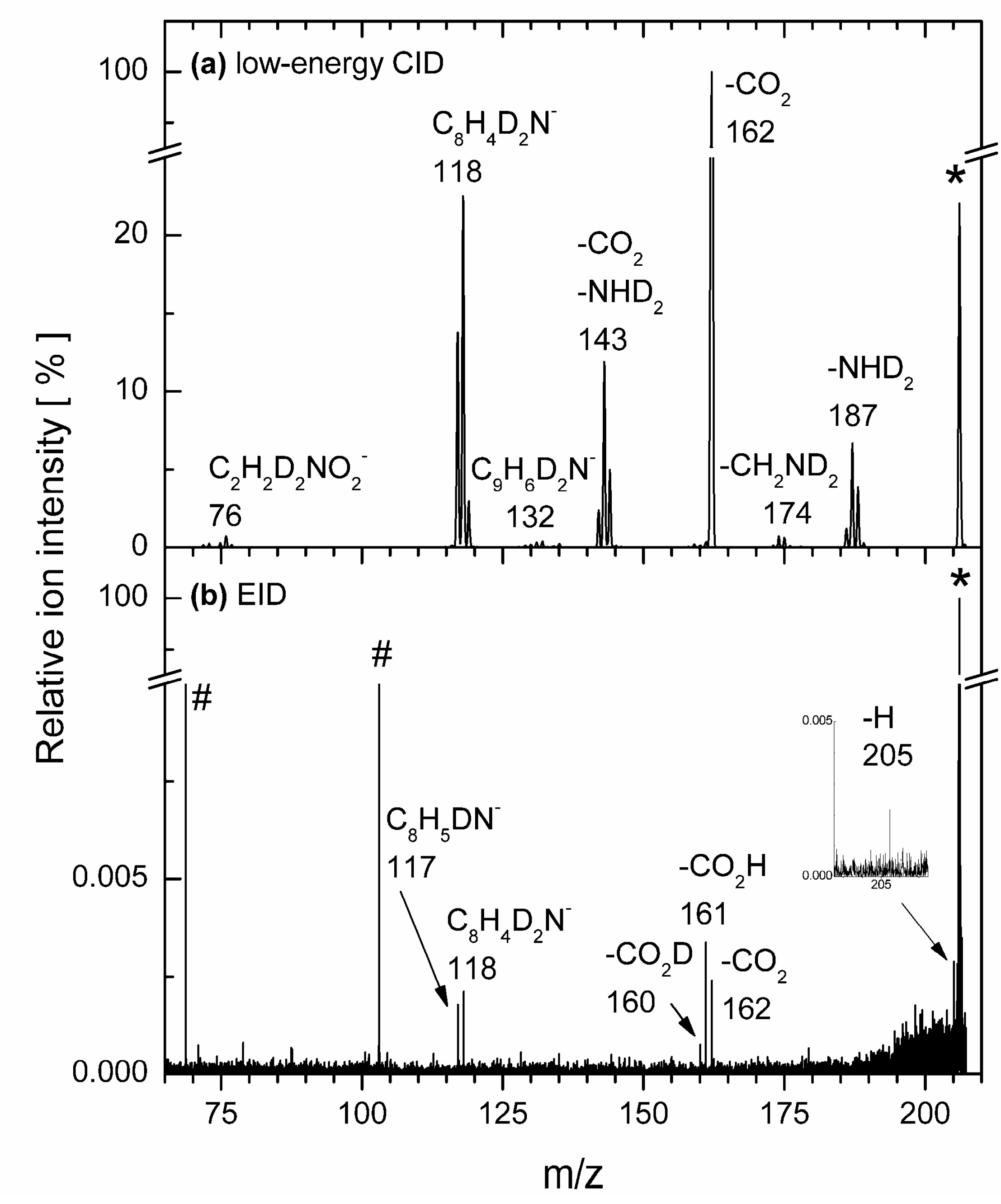

(a) low-energy CID
100
20
10
0
$C_8H_4D_2N^-$
118
-CO_2
162
-CO_2
-NHD_2
143
-NHD_2
187
*
$C_2H_2D_2NO_2^-$
76
$C_9H_6D_2N^-$
132
-CH_2ND_2
174
(b) EID
100
0.005
0.000
#
#
*
-H
205
0.005
0.000
205
$C_8H_5DN^-$
117
$C_8H_4D_2N^-$
118
-CO_2H
161
-CO_2D
160
-CO_2
162
75
100
125
150
175
200
Relative ion intensity [%]
m/z

Figure 3:

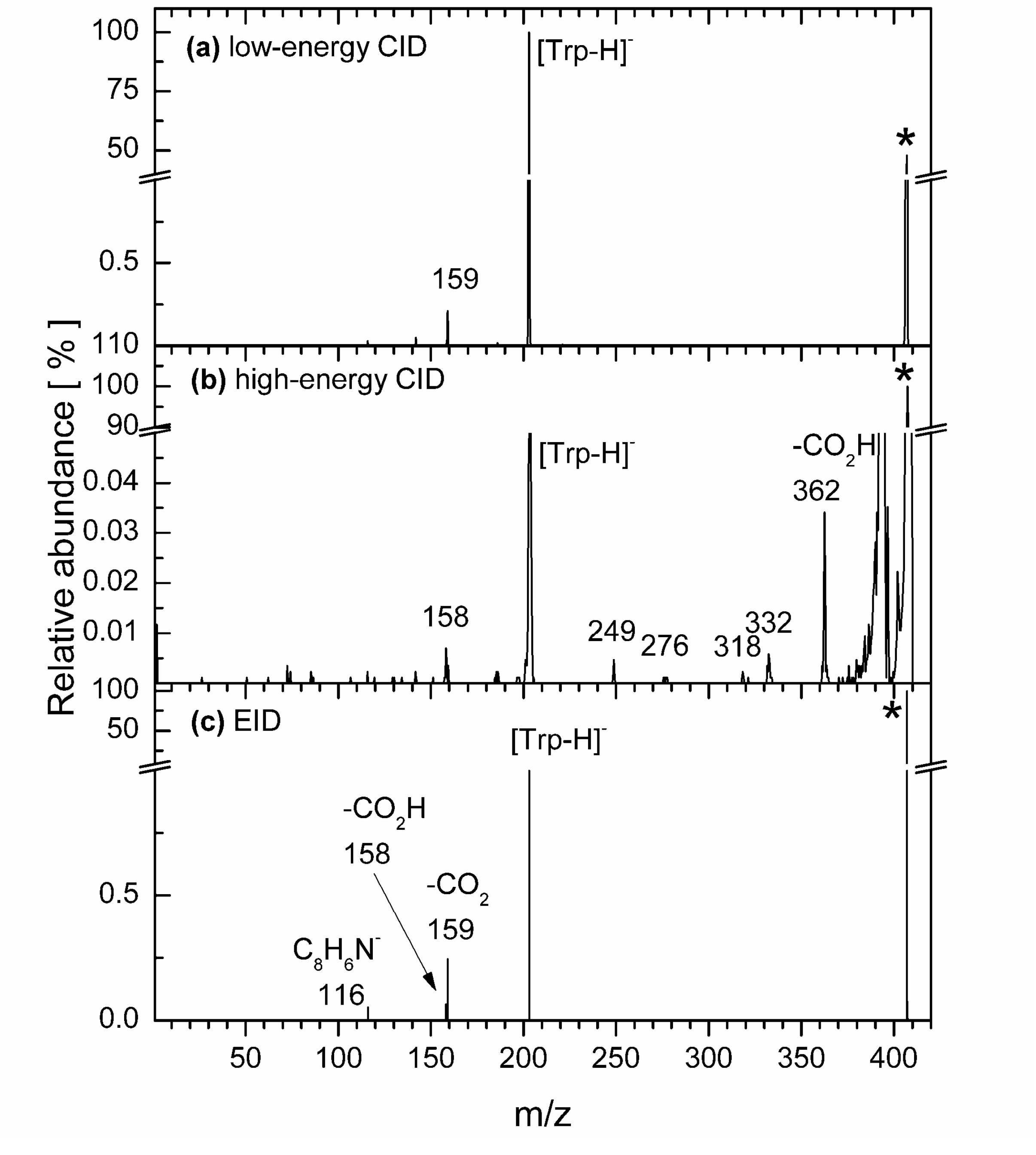

(a) low-energy CID
[Trp-H]⁻
*
159
(b) high-energy CID
[Trp-H]⁻
*
-CO₂H
362
158
249
276
318
332
(c) EID
[Trp-H]⁻
*
-CO₂H
158
-CO₂
159
C₈H₆N⁻
116
Relative abundance [%]
m/z
100
75
50
0.5
110
90
0.04
0.03
0.02
0.01
0.0
50
100
150
200
250
300
350
400

Figure 4:

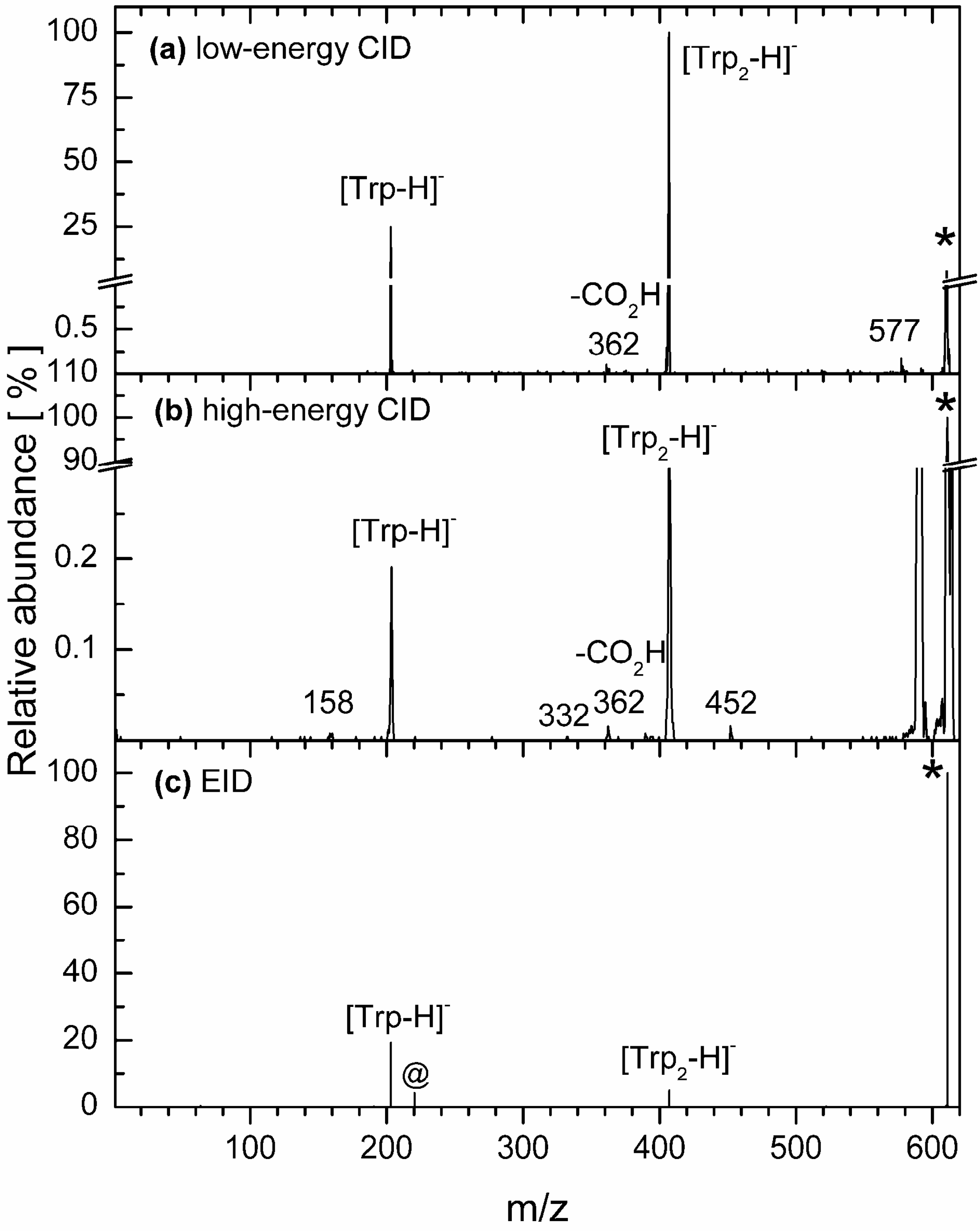
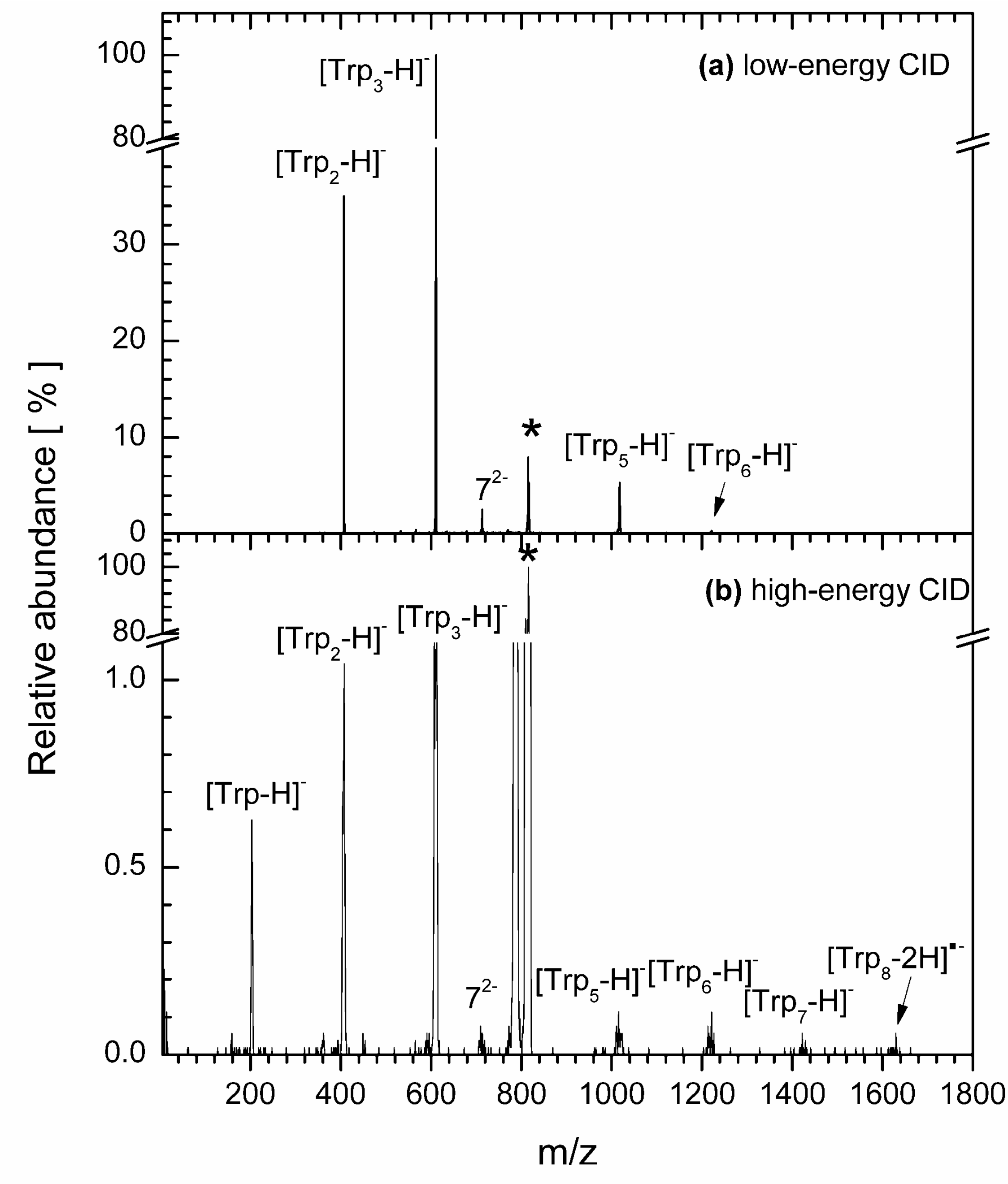

(a) low-energy CID
[Trp$_2$-H]$^-$
[Trp-H]$^-$
-CO$_2$H
362
577
*
(b) high-energy CID
[Trp$_2$-H]$^-$
[Trp-H]$^-$
-CO$_2$H
158
332
362
452
*
(c) EID
[Trp-H]$^-$
@
[Trp$_2$-H]$^-$
*
Relative abundance [%]
100
75
50
25
0.5
110
100
90
0.2
0.1
100
80
60
40
20
0
100
200
300
400
500
600
m/z

Figure 5:


(a) low-energy CID
[Trp$_3$-H]$^-$
[Trp$_2$-H]$^-$
7^{2-}
*
[Trp$_5$-H]$^-$
[Trp$_6$-H]$^-$
(b) high-energy CID
*
[Trp$_2$-H]$^-$
[Trp$_3$-H]$^-$
[Trp-H]$^-$
7^{2-}
[Trp$_5$-H]$^-$
[Trp$_6$-H]$^-$
[Trp$_7$-H]$^-$
[Trp$_8$-2H]$^{\bullet-}$
Relative abundance [%]
m/z
0
10
20
30
80
100
0.0
0.5
1.0
80
100
200
400
600
800
1000
1200
1400
1600
1800