Structural analysis (CH-314)

Week 4

Problems and Solutions

Problem 1. Ions of mass m are analyzed by an Orbitrap mass spectrometer. How much the frequency of the axial oscillations of the doubly charged ions is higher than that of the singly charged?

Solution:

Frequency of axial oscillations is given by the following equation:

$$\omega_z = \sqrt{\frac{k}{m/q}}$$

Thus, the ratio of the frequencies for the doubly and singly charged ions is:

$$\frac{\omega_z(+2)}{\omega_z(+1)} = \sqrt{\frac{2}{1}} = 1.41$$

Problem 2. Determine the nominal, monoisotopic, and average masses of peptide bradykinin (H-RPPGFSPFR-OH). Monoisotopic masses of the elements: m(^{1}H) = 1.007825 Da, m(^{12}C) = 12.00000 Da, m(^{14}N) = 14.00307 Da, m(^{16}O) = 15.99491 Da. Average masses of the elements: m(H) = 1.00794 Da, m(C) = 12.011 Da, m(N) = 14.00674 Da, m(O) = 15.9994 Da. What are the protonation/deprotonation sites in this peptide? Calculate the mass-to-charge ratio of protonated/deprotonated bradykinin ions.

Solution:

First, one has to calculate the number of atoms of each element in the peptide:

Residue	Side chain	H	C	N	O
N-Terminus	-	1	0	0	0
Arginine (R)	$C_4H_{10}N_3$	12	6	4	1
Proline (P)	C_3H_5	7	5	1	1
Proline (P)	C_3H_5	7	5	1	1
Glycine (G)	H	3	2	1	1
Phenylalanine (F)	C_7H_7	9	9	1	1
Serine (S)	CH_3O	5	3	1	2
Proline (P)	C_3H_5	7	5	1	1
Phenylalanine (F)	C_7H_7	9	9	1	1
Arginine (R)	$C_4H_{10}N_3$	12	6	4	1
C-Terminus	-	1	0	0	1
Total		73	50	15	11

Thus, the elemental composition of the peptide is $H_{73}C_{50}N_{15}O_{11}$. To calculate the nominal, monoisotopic, and average masses of the peptide, one sums up the respective atomic masses multiplied by the number of atoms of each type:

$$m_{nominal} = 73 \cdot 1 \text{ Da} + 50 \cdot 12 \text{ Da} + 15 \cdot 14 \text{ Da} + 11 \cdot 16 \text{ Da} = 1059 \text{ Da}$$

$$m_{monoisotopic} = 73 \cdot 1.007825 \text{ Da} + 50 \cdot 12.00000 \text{ Da} +$$

$$+ 15 \cdot 14.00307 \text{ Da} + 11 \cdot 15.99491 \text{ Da} = 1059.561285 \text{ Da}$$

$$m_{average} = 73 \cdot 1.00794 \text{ Da} + 50 \cdot 12.011 \text{ Da} +$$

$$+ 15 \cdot 14.00674 \text{ Da} + 11 \cdot 15.9994 \text{ Da} = 1060.22412 \text{ Da}$$

There is only one deprotonation site, namely, the C-terminus ($COOH \rightleftharpoons COO^- + H^+, pK_a = 2.17$) while the protonation sites are the N-terminus ($NH_3^+ \rightleftharpoons NH_2 + H^+, pK_a = 9.04$) and the side chains of arginines ($-(CH_2)_3 - NH - C(NH_2)^+ \rightleftharpoons -(CH_2)_3 - NH - C(NH_2) = NH + H^+, pK_a = 12.48$).

The mass-to-charge ratios of the bradykinin ions are calculated as follows:

$$m/z([M+H]^+) = \frac{1059.561285 \text{ Da} + 1.007276 \text{ Da}}{1} = 1060.568561 \text{ Th}$$

$$m/z([M+2H]^{2+}) = \frac{1059.561285 \text{ Da} + 2 \cdot 1.007276 \text{ Da}}{2} = 530.7879185 \text{ Th}$$

$$m/z([M+3H]^{3+}) = \frac{1059.561285 \text{ Da} + 3 \cdot 1.007276 \text{ Da}}{3} = 354.194371 \text{ Th}$$

$$m/z([M-H]^{-}) = \frac{1059.561285\ \mathrm{Da} - 1.007276\ \mathrm{Da}}{1} = 1058.554009\ \mathrm{Th}$$

Problem 3. Which resolution one needs to resolve monoisotopic peaks of triply protonated peptides H-RSHRGHR-OH and H-RVMRGMR-OH?

Solution:

To calculate the mass of a peptide one sums up the masses of its residues and adds the mass of a water:

$$m(H\text{-}RSHRGHR\text{-}OH) = m\big(R_{Arg} + C_2H_2NO\big) + m(R_{Ser} + C_2H_2NO) +$$

$$+ m(R_{His} + C_2H_2NO) + m\big(R_{Arg} + C_2H_2NO\big) + m\big(R_{Gly} + C_2H_2NO\big) + m(R_{His} + C_2H_2NO) +$$

$$+ m\big(R_{Arg} + C_2H_2NO\big) + m(H_2O) = 156.101111\ \mathrm{Da} + 87.032028\ \mathrm{Da} +$$

$$+ 137.058912\ \mathrm{Da} + 156.101111\ \mathrm{Da} + 57.021464\ \mathrm{Da} + 137.058912\ \mathrm{Da} +$$

$$+ 156.101111\ \mathrm{Da} + 18.010565\ \mathrm{Da} = 904.485214\ \mathrm{Da}$$

$$m(H\text{-}RVMRGMR\text{-}OH) = m\big(R_{Arg} + C_2H_2NO\big) + m(R_{Val} + C_2H_2NO) +$$

$$+ m(R_{Met} + C_2H_2NO) + m\big(R_{Arg} + C_2H_2NO\big) + m\big(R_{Gly} + C_2H_2NO\big) + m(R_{Met} + C_2H_2NO) +$$

$$+ m\big(R_{Arg} + C_2H_2NO\big) + m(H_2O) = 156.101111\ \mathrm{Da} + 99.068414\ \mathrm{Da} +$$

$$+ 131.040485\ \mathrm{Da} + 156.101111\ \mathrm{Da} + 57.021464\ \mathrm{Da} + 131.040485\ \mathrm{Da} +$$

$$+ 156.101111\ \mathrm{Da} + 18.010565\ \mathrm{Da} = 904.484746\ \mathrm{Da}$$

Therefore, the mass-to-charge ratios of the triply protonated peptides are:

$$m/z([RSHRGHR + 3H]^{3+}) = \frac{904.485214\ \mathrm{Da} + 3 \cdot 1.007276\ \mathrm{Da}}{3} = 302.502347\ \mathrm{Th}$$

$$m/z([RVMRGMR + 3H]^{3+}) = \frac{904.484746\ \mathrm{Da} + 3 \cdot 1.007276\ \mathrm{Da}}{3} = 302.502191\ \mathrm{Th}$$

Then, the required resolution is estimated as follows:

$$R = 1.8 \cdot \frac{m/z}{\Delta m/z} = 1.8 \cdot \frac{\frac{1}{2} \cdot (302.502347\ \mathrm{Th} + 302.502191\ \mathrm{Th})}{302.502347\ \mathrm{Th} - 302.502191\ \mathrm{Th}} \approx 3.5 \cdot 10^6$$

Problem 4. Doubly protonated peptide substance P (H-RPKPQQFFGLM-OH) is subjected to collision-induced dissociation (CID). Calculate at what m/z you would expect to see the following fragment ions: a) b_5^+, b_6^+; b) y_4^+, y_6^+; c) b_4^{2+}.

Solution:

The general formulas for calculating the mass-to-charge ratio of b- and y-ions are:

$$m/z(b_k^{q+}) = \frac{\sum_{i=1}^{k-1} m(R_i + C_2H_2NO) + m(R_k) + m(C_2H_2NO) + q \cdot m(H^+)}{q}$$

$$m/z(y_k^{q+}) = \frac{\sum_{i=1}^{k-1} m(R_i + C_2H_2NO) + m(R_k) + m(C_2H_4NO_2) + q \cdot m(H^+)}{q}$$

Therefore, the m/z of the b_5^+ fragment, for instance, is (in this case q=1):

$$m/z(b_5^+) = m\left(R_{Arg} + C_2H_2NO\right) + m(R_{Pro} + C_2H_2NO) + m\left(R_{Lys} + C_2H_2NO\right) +$$

$$+ m(R_{Pro} + C_2H_2NO) + m(R_{Gln}) + m(C_2H_2NO) + m(H^+) = 607.367456\ \mathrm{Th}$$

Then, to calculate the m/z of the b_6^+ fragment, one just adds the mass of the sixth residue to the m/z of the b_5^+ fragment:

$$m/z(b_6^+) = m/z(b_5^+) + m(R_{Gln} + C_2H_2NO) = 735.426034\ \mathrm{Th}$$

For y-ions, one sums up the masses of residues starting from the C-terminus:

$$m/z(y_4^+) = m(R_{Met} + C_2H_2NO) + m(R_{Leu} + C_2H_2NO) + m\left(R_{Gly} + C_2H_2NO\right) +$$

$$+ m(R_{Phe}) + m(C_2H_4NO_2) + m(H^+) = 467.232267\ \mathrm{Th}$$

Then, to calculate the m/z of the y_6^+ fragment, one just adds the mass of the fifth and sixth residues to the m/z of the y_4^+ fragment:

$$m/z(y_6^+) = m/z(y_4^+) + m(R_{Phe} + C_2H_2NO) + m(R_{Gln} + C_2H_2NO) = 742.359259\ \mathrm{Th}$$

To calculate the m/z of the b_4^{2+} fragment, one may subtract the mass of the fifth residue from the m/z of the b_5^+ fragment, add the mass of a proton and divide by 2:

$$m/z(b_4^{2+}) = \frac{m/z(b_5^+) - m(R_{Gln} + C_2H_2NO) + m(H^+)}{2} = 240.158077\ \mathrm{Th}$$

Problem 5. A doubly protonated peptide was subjected to CID MS/MS. The obtained mass spectrum contains peaks given below. Assuming the mass accuracy of 5 ppm, perform *de novo* sequencing:

182.081 Th
241.075 Th
338.182 Th
370.118 Th
439.230 Th
501.158 Th
526.262 Th
570.252 Th (precursor ion)
614.242 Th
639.346 Th
701.274 Th
770.386 Th
802.322 Th
899.429 Th
958.423 Th
1036.488 Th

Solution:

This fragmentation mass spectrum comprises fragments, which have m/z higher than that of the precursor ion. This means, that these ions are singly protonated (the precursor is doubly protonated). The m/z of the singly protonated precursor ion is:

$$m/z([M+H]^{+}) = 2 \cdot m/z([M+2H]^{2+}) - m(H^{+}) = 1139.497324\ \mathrm{Th}$$

There is no such peak in the spectrum, however, one can calculate the difference between this m/z and the highest m/z found in the spectrum:

$$\Delta = 1139.497324\ \mathrm{Th} - 1036.488\ \mathrm{Th} = 103.009324\ \mathrm{Th}$$

The only residue which has the same nominal mass is cysteine and the relative difference between these two masses lies within the experimental accuracy:

$$\delta m = \frac{103.009324\ \mathrm{Da} - 103.009185\ \mathrm{Da}}{103.009324\ \mathrm{Da}} = 1.35\ \mathrm{ppm}$$

Thus, the peak at 1036.488 Th can be tentatively assigned to the y_{N-1} fragment, where N is the total number of amino acids in the peptide (note that the mass of the y_N fragment is formally the same as of the precursor peptide).

Then, consider the mass difference between the peak at 1036.488 Th and the previous two peaks in the list:

$$\Delta = 1036.488\ \mathrm{Th} - 958.4233\ \mathrm{Th} = 78.0647\ \mathrm{Th}$$

$$\Delta = 1036.488\ \mathrm{Th} - 899.4291\ \mathrm{Th} = 137.0589\ \mathrm{Th}$$

The first difference does not correspond to the mass of any residue, while the second difference equals the mass of a histidine residue, which indicates the presence of histidine in the peptide.

By walking through the list of fragments, one can deduce the following:

1. The difference $\Delta = 899.4291\ \text{Th} - 802.3222\ \text{Th} = 97.1069\ \text{Th}$ cannot be assigned to a proline residue (although it has the same nominal mass), because

$$\delta m \approx \frac{97.1069\ \text{Th} - 97.052764\ \text{Th}}{97\ \text{Th}} \approx 558\ \text{ppm} \gg 5\ \text{ppm}$$

   but the difference $\Delta = 899.4291\ \text{Th} - 770.3865\ \text{Th} = 129.0426\ \text{Th}$ perfectly matches the mass of a glutamine residue.
2. The difference $\Delta = 770.3865\ \text{Th} - 701.2745\ \text{Th} = 69.112\ \text{Th}$ does not correspond to any residue, but the difference $\Delta = 770.3865\ \text{Th} - 639.3461\ \text{Th} = 131.0404\ \text{Th}$ matches the mass of a methionine residue.
3. The difference $\Delta = 639.3461\ \text{Th} - 614.2425\ \text{Th} = 25.1036\ \text{Th}$ is too small to be the mass of a residue, but the difference $\Delta = 639.3461\ \text{Th} - 526.262\ \text{Th} = 113.0841\ \text{Th}$ matches well the mass of a leucine or isoleucine residue. This also means that the peak at 526.262 Th, although it has m/z lower than that of the doubly protonated precursor ion, is a singly protonated fragment. Therefore, one should continue looking for singly protonated fragments.
4. The difference $\Delta = 526.262\ \text{Th} - 501.1585\ \text{Th} = 25.1035\ \text{Th}$ is too small to be the mass of a residue (assuming z = 1), but the difference $\Delta = 526.262\ \text{Th} - 439.23\ \text{Th} = 87.032\ \text{Th}$ matches well the mass of a serine residue.
5. The difference $\Delta = 439.23\ \text{Th} - 370.118\ \text{Th} = 69.112\ \text{Th}$ does not correspond to any residue. The difference $\Delta = 439.23\ \text{Th} - 338.1823\ \text{Th} = 101.0477\ \text{Th}$ matches well the mass of a threonine residue.
6. The difference $\Delta = 338.1823\ \text{Th} - 241.0754\ \text{Th} = 97.1069\ \text{Th}$ does not correspond to proline residue, because $m \approx \frac{97.1069\ \text{Da} - 97.052764\ \text{Da}}{97\ \text{Da}} \approx 558\ ppm \gg 5\ ppm$. The difference $\Delta = 338.1823\ \text{Th} - 182.0812\ \text{Th} = 156.1011\ \text{Th}$ however matches the mass of an arginine residue.
7. The peak at 182.0812 Th can be a singly protonated y_1 fragment. If this is the case, then its m/z is $m/z(y_1) = m(R_1) + m(C_2H_4NO_2) + m(H^+)$ and, therefore, the mass of the residue side chain is $m(R_1) = m/z(y_1) - m(C_2H_4NO_2) - m(H^+) = 107.049721\ \text{Da}$, which corresponds to the mass of a tyrosine side chain.

Thus, one may tentatively assign this sequence of y-fragments to the following peptide:

$$Cys - His - Glu - Met - Leu/Ile\ -\ Ser - Thr - Arg - Tyr$$

To confirm the proposed sequence, one should assign the other peaks in the fragmentation mass spectrum (they should correspond to the b-fragments):

The peak at 958.4233 Th may be a b_{N-1} fragment. The mass of the b_N fragment formally equals the mass of the entire peptide after a neutral water loss. Thus, difference $\Delta = 1139.497324\ \text{Th} - 958.4233\ \text{Th} = 181.074024\ \text{Th}$ should be equal to the mass of tyrosine residue plus the mass of a water molecule, i.e., $163.063329\ \text{Da} + 18.010565\ \text{Da} = 181.073894\ \text{Da}$, which is true within the experimental mass accuracy.

The differences $\Delta = 958.4233\ \text{Th} - 802.3222\ \text{Th} = 156.1011\ \text{Th}$, $\Delta = 802.3222\ \text{Th} - 701.2745\ \text{Th} = 101.0477\ \text{Th}$, $\Delta = 701.2745\ \text{Th} - 614.2425\ \text{Th} = 87.032\ \text{Th}$, $\Delta = 614.2425\ \text{Th} - 501.1585\ \text{Th} = 113.084\ \text{Th}$, $\Delta = 501.1585\ \text{Th} - 370.118\ \text{Th} = 131.0405\ \text{Th}$, $\Delta = 370.118\ \text{Th} - 241.0754\ \text{Th} = 129.0426\ \text{Th}$ correspond to the masses of arginine, threonine, serine, leucine/isoleucine, methionine, and glutamine residues, respectively.

Finally, for the proposed peptide sequence, the peak at 241.0754 Th should correspond to the b_2 fragment. Its m/z can be directly calculated: $m/z(b_2^+) = m\left(R_{Cys} + C_2H_2NO\right) + m(R_{His}) + m(C_2H_2NO) + m(H^+) = 241.075373\ \mathrm{Th}$, which is consistent with the experimental m/z of the peak.

These considerations validate the proposed peptide sequence. Thus, the peptide is either

$$H - CHEMLSTRY - OH$$

or

$$H - CHEMISTRY - OH$$

and the assignment of the peaks in the fragmentation mass spectrum is:

y_1	182.0812 Th
b_2	241.0754 Th
y_2	338.1823 Th
b_3	370.118 Th
y_3	439.23 Th
b_4	501.1585 Th
y_4	526.262 Th
$[M+2H]^{2+}$	570.2523 Th
b_5	614.2425 Th
y_5	639.3461 Th
b_6	701.2745 Th
y_6	770.3865 Th
b_7	802.3222 Th
y_7	899.4291 Th
b_8	958.4233 Th
y_8	1036.488 Th