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Analysis of Tyrosine and its Halogenated Forms and Tripeptide –Metal Ion Clusters Using Mass Spectrometry

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GRADUATE RESEARCH PROJECT

Chapter- I

Calculation of proton affinities of tyrosine and its halogenated forms using mass spectrometry

Research Advisor: Dr. Joong-Won Shin

Submitted by: Bharathi Gannamani

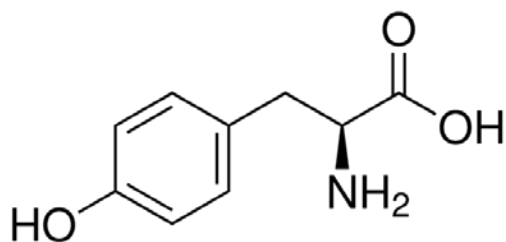


ABSTRACT:

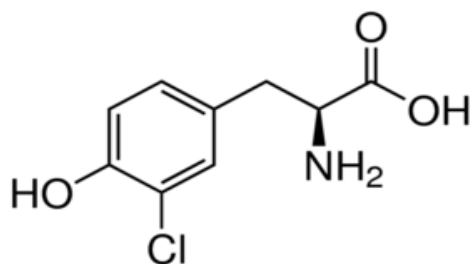
The aim of this research project was to measure the differences in proton affinities of tyrosine and its halogenated forms, 3-chlorotyrosine and 3-iodotyrosine. Tandem mass spectrometry was used here to measure the proton affinities. These amino acids were formed as protonated dimers with amino acids of known proton affinity and were introduced into the instrument through electrospray ionization (ESI). Based on the intensities of the protonated amino acids appeared in the mass spectrum when the dimer was isolated and fragmented, the proton affinities of the sample amino acids were calculated.

INTRODUCTION:

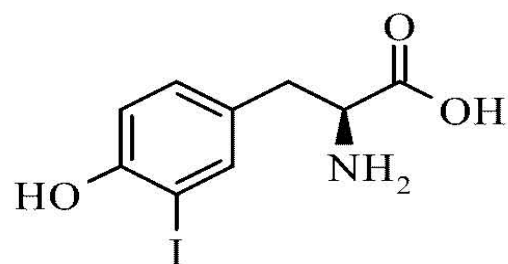
Mass spectrometry is a technique in which the identification is based on the mass to charge (m/z) ratio. The samples are introduced into the instrument through ESI. In ESI, the sample is sprayed through a fine capillary syringe which is held at high voltage. This is a positive ion mode, so only positively charged, protonated ions are detected. These protonated ions were isolated in an ion trap and are fragmented by collision induced dissociation (CID).



tyrosine



3-chlorotyrosine



[3]

3-iodotyrosine

EXPERIMENTAL SECTION:

- The solvent used for this experiment was a solution of 80% water, 20% methanol, 1% acetic acid.
- The sample amino acids used were tyrosine, 3-chlorotyrosine, 3-iodotyrosine .
- The reference amino acids used here were threonine (Thr), phenyl alanine (Phe), isoleucine (Ile) and methionine (Met).
- When the samples were prepared, 5-6 drops of HCl were added for better solubility.
- All the amino acid samples were prepared with a concentration of 0.050M and are analyzed by introducing the samples into mass spectrometer using a syringe pump at a flow rate of 0.30 ml/hr.
- The nebulizer gas N₂ helps in electrospray by desolvating the ions and the desolvated ions were trapped in an ion trap. The buffer gas used here is the He gas.
- The protonated forms of the amino acids were identified and were fragmented with amplitude of 0.35V.
- The voltage for fragmentation was kept the same for all the samples and was the minimum voltage for obtaining sufficient product ions from the isolated protonated dimer.
- The isolation width was maintained at m/z=2.0. The other parameters used throughout the experiment are nebulizer gas 40 psi, dry gas 5.0 l/min and dry temperature at 250°C.
- The experiment was repeated four times for reproducibility.
- Kinetic method was used for the calculation of proton affinity values of the amino acids. The formula used was

$$\ln k/k_i = 1/RT_{\text{eff}}(PA-PA_i) \text{ ----- (1)}$$

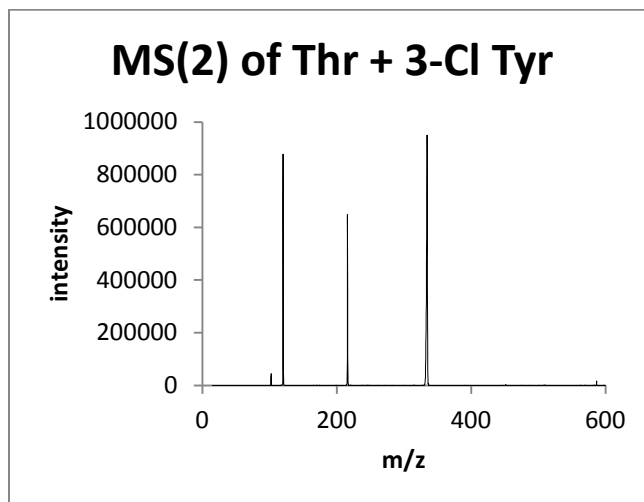
Where k and k_i are rate constants, T_{eff} is the effective temperature at which the amino acid dimer fragments, R is the ideal gas constant, PA_i is the proton affinity of the reference compound and PA is the proton affinity of the unknown compound.

- A plot was drawn for each amino acid based on the above equation, T_{eff} was calculated from the slope and proton affinity of the analyte (PA) was calculated from the intercept of the line equation.

RESULTS AND DISCUSSION:

- A dimer was formed combining each unknown amino acid with each reference amino acid.
- For example, 3-chlorotyrosine was mixed with each of the reference amino acids, Met, Thr, Phe and Ile. Thus four protonated dimers were formed for each amino acid with a combined concentration of 0.025M.
- Each dimer sample was injected using a syringe pump into the mass spectrometer through electrospray ionization (ESI).

- I/I_i are assumed to be the same as the ratio of rate constants k/k_i based on the kinetic method.
- The dimer ion on the mass spectrum was identified and fragmented, the intensity of both the reference and unknown amino acid were taken and $\ln k/k_i$ was calculated.
- The proton affinity (PA) values of the reference amino acids were taken from the reference.^[2]
- A plot was made by taking PA values on the x-axis and $\ln k/k_i$ on the y-axis.



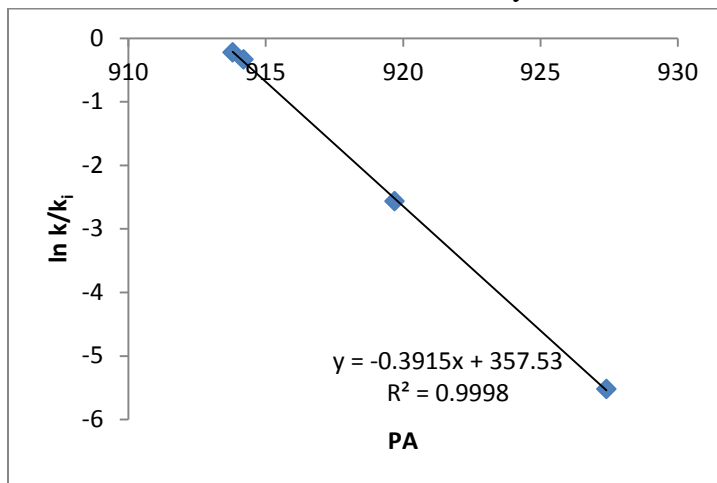
AA	m/z	Intensity
Cl-Tyr(k)	216	647834
Thr(k _i)	120,102	905973

$$\ln k/k_i = \frac{647834}{905973} = -0.335375.$$

- In the similar way, $\ln k/k_i$ values were calculated for all the other three dimer samples for the amino acid 3-chlorotyrosine.

AA	PA(x-axis) ^[2]	$\ln k/k_i$ (y-axis)
Met	927.4	-5.52271
Thr	914.2	-0.33538
Phe	919.7	-2.56665
Ile	913.8	-0.22737

- A plot is drawn with PA values on x-axis and $\ln k/k_i$ on y-axis.



- The equation (1) was solved in order to match with the line equation of the plot above.

$$\ln k/k_i = -1/RT_{\text{eff}}(\text{PA}_i) + 1/RT_{\text{eff}}(\text{PA}) \text{ ----- (2)}$$

$$y = -0.3915x + 357.53 \text{ ----- (3)}$$

- From the equations (2) and (3), we can say that

$$1/RT_{\text{eff}} = 0.3915 \text{ mol/kJ} \text{ ----- (4) and}$$

$$1/RT_{\text{eff}}(\text{PA}) = 357.53 \text{ ----- (5)}$$

- Effective temperature T_{eff} was calculated from the equation (4), which is

$$T_{\text{eff}} = 307.2262 \text{ K}$$

- Proton affinity of the unknown amino acid, 3-chlorotyrosine was calculated from the equation (5).

$$\text{PA} = 913.2312 \text{ kJ}\cdot\text{mol}^{-1}$$

- The values of effective temperature and proton affinity were calculated for all the three amino acids in the same manner.

Amino acid	$T_{\text{eff}}(\text{K})$	PA ($\text{kJ}\cdot\text{mol}^{-1}$)
3-chlorotyrosine	307.2262	913.2312
3-iodotyrosine	299.4996	917.7789
tyrosine	355.6447	922.6789

- Similarly, the process was repeated three more times to check the reproducibility. The results of all the sets along with the standard deviation are tabulated below.

Amino acid	$T_{\text{eff}}(\text{K})$				$\text{PA} (\text{kJ}\cdot\text{mol}^{-1})$				
	Set 1	Set 2	Set 3	Set 4	Set 1	Set 2	Set 3	Set 4	Std. dev.
3-chlorotyrosine	307.2	335.3	299.6	324.6	913.2	913.0	913.2	912.8	0.2
3-iodotyrosine	299.5	307.3	305.4	293.5	917.8	918.0	918.0	918.0	0.1
tyrosine	355.6	367.2	353.1	352.6	922.7	923.0	922.5	922.5	0.1

CONCLUSION:

Upon halogenation, the proton affinity of tyrosine decreases. 3-chlorotyrosine has a low proton affinity of $913.2312 \text{ kJ}\cdot\text{mol}^{-1}$ and 3-iodotyrosine has a proton affinity of $917.9612 \text{ kJ}\cdot\text{mol}^{-1}$, which is higher than 3-chlorotyrosine and lower than tyrosine. Electronegativity might be the reason for the difference in proton affinities of 3-chlorotyrosine and 3-iodotyrosine as chlorine is more electronegative than iodine. When a halogen presents on the aromatic ring of the amino acids 3-chlorotyrosine and 3-iodotyrosine, it makes the other half of the molecule partially positive. This results in the decrease of proton affinity of these compared to tyrosine.

REFERENCES:

- [1] K.B. Green-Church, P.A. Limbach, J. Am. Soc. Mass Spectrom. 11 (2000) 24.
- [2] C. Afonso, F. Modeste, P. Brenton, F. Fournier, J.-C. Tabet, Eur. J. Mass Spectrom. 6 (2000) 443.
- <http://www.sigmaaldrich.com/catalog/product>

GRADUATE RESEARCH PROJECT

Chapter- II

Analysis of tripeptide –metal ion clusters using mass spectrometry

Research Advisor: Dr. Joong-Won Shin

Submitted by: Bharathi Gannamani



ABSTRACT:

The research project was on mass spectrometry of mixtures of peptides and metals. A tripeptide was mixed with different metals of certain concentration and were introduced into the instrument through electrospray ionization (ESI) using a syringe pump. Later, the $[\text{metal+peptide-H}]^+$ of the tripeptide and the metal ion was isolated and fragmented. This shows how the tripeptide fragments specific to each of the doubly charged metal ion.

INTRODUCTION:

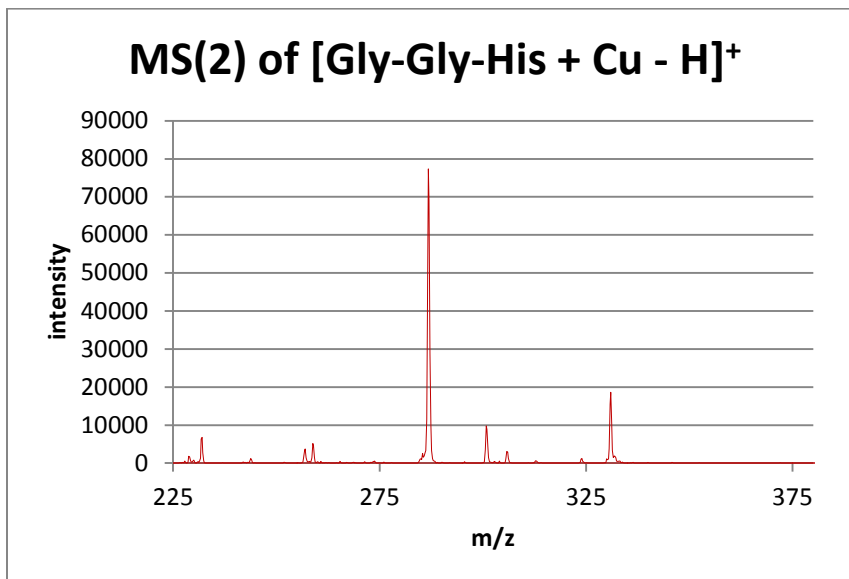
Mass spectrometry is a technique in which the identification is based on the mass to charge (m/z) ratio. The samples are introduced into the instrument through ESI. In ESI, the sample is sprayed through a fine capillary syringe which is held at high voltage. When the metal is combined with the tripeptide and is introduced into the mass spectrometer a cluster of $[\text{metal+peptide-H}]^+$ forms which is isolated in an ion trap and fragmented using the collision induced dissociation (CID).

EXPERIMENTAL SECTION:

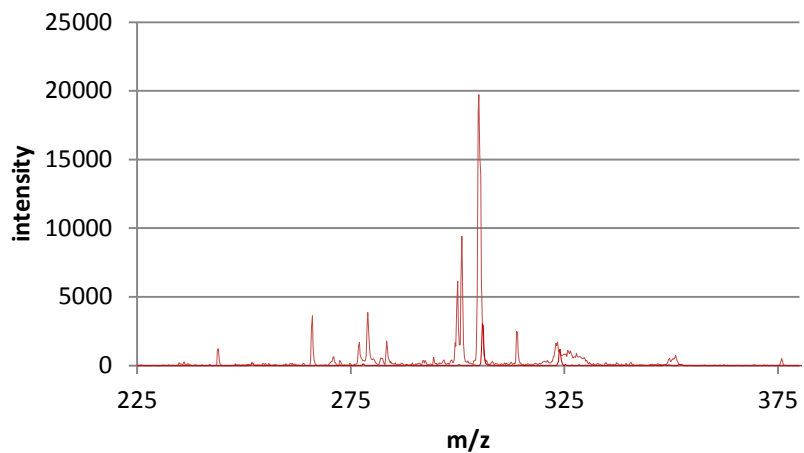
- The solvent used in this experiment was a 50:50 ratio of water and methanol.
- The tripeptide used was Gly-Gly-His and the metal compounds used were $\text{C}_6\text{H}_{26}\text{Cl}_2\text{N}_6\text{NiO}\cdot x\text{H}_2\text{O}$, $\text{Zn}(\text{CH}_3\text{COO})_2$, $\text{Cu}(\text{CH}_3\text{COO})_2$, $\text{Mn}(\text{CH}_3\text{COO})_2$ and Cl_2Fe .
- The tripeptide and the metal solutions were prepared with a concentration of 0.005 M each.
- The tripeptide solution was combined with each metal solution in equal proportion which makes the concentration of each to 0.0025M.
- All the samples were introduced into the mass spectrometer one after one using a syringe pump at a flow rate of 0.30 ml/hr.
- The nebulizer gas used here was N_2 , which helps in electro spray by desolvating the ions and then the desolvated ions were trapped in an ion trap. The buffer gas was He gas.
- $[\text{metal+peptide-H}]^+$ was isolated and fragmented with a specific amplitude.
- The amplitude varies and was based on the fragmentation of the metal ion from the isolated parent ion.
- The isolation width was maintained at $m/z=2.0$. The other parameters used through out the experiment were nebulizer gas 40 p.s.i., dry gas 7.0 l/min and dry temperature at 300°C .
- The experiment was repeated three times for reproducibility.

RESULTS AND DISCUSSION:

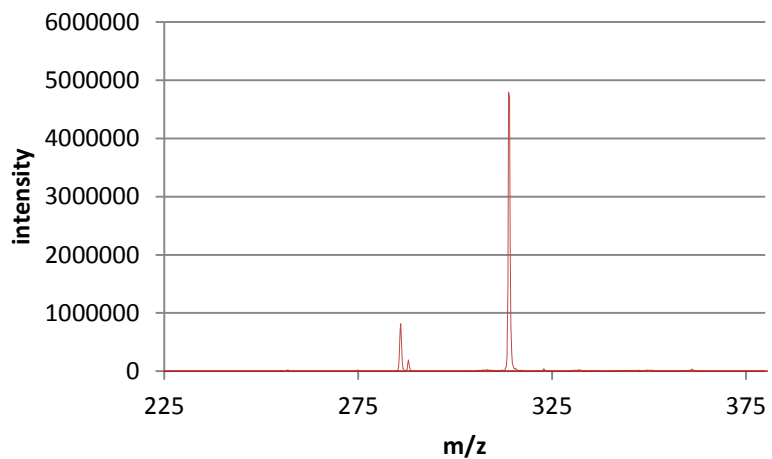
- A series of samples were formed by combining the tripeptide with each of the metal solution. Thus there were five samples prepared with a combined concentration of 0.0025 M.
- Each sample was introduced into the mass spectrometer using a syringe pump through electrospray ionization (ESI).
- When the sample with tripeptide and Fe was injected, the intensity of $[\text{Fe}+\text{GGH}-\text{H}]^+$ was very low on the mass spectrum, so the concentration of Fe was doubled which is 0.010 M whereas the concentration of tripeptide was not changed.
- The deprotonated ion was isolated and fragmented. The pattern of the tripeptide responding to each metal was identified.
- The CID results are below:

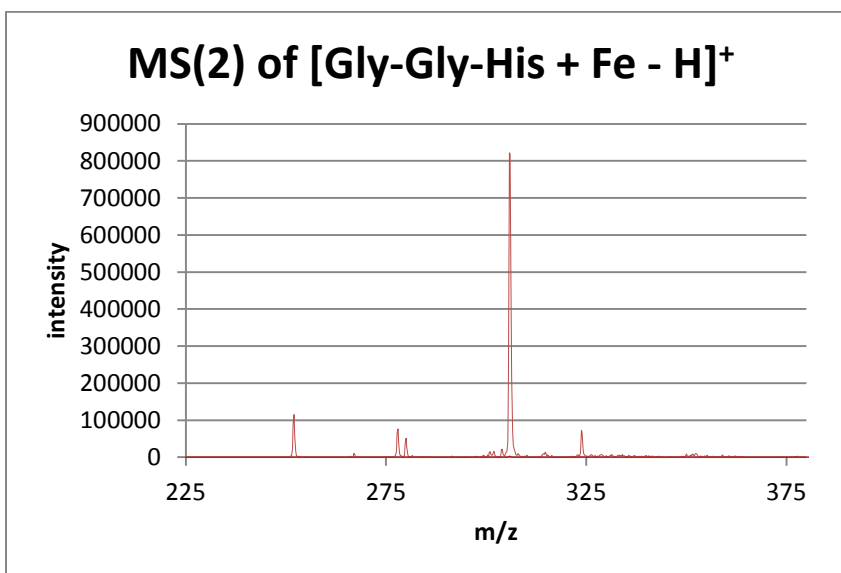
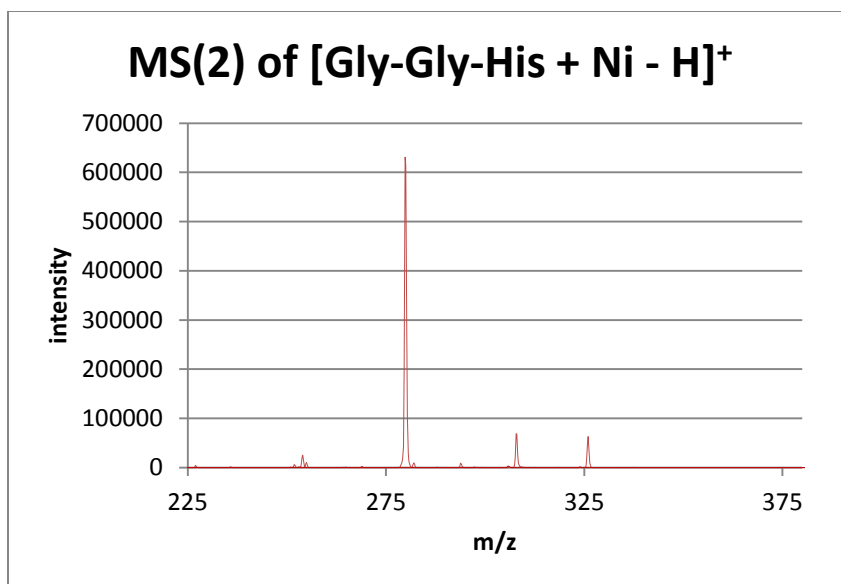


MS(2) of [Gly-Gly-His + Mn - H]⁺



MS(2) of [Gly-Gly-His + Zn - H]⁺





Result of CID	-H ₂ O		-CO ₂ / -CO ₂ , -H ₂	
	m/z ratio	intensity	m/z ratio	intensity
[GGH + Cu-H] ⁺	-	-	287	77391
[GGH + Mn-H] ⁺	305	19736	279	3858
[GGH + Zn-H] ⁺	314	4698140	288	192838
[GGH + Ni-H] ⁺	308	68882	280	615396
[GGH + Fe-H] ⁺	306	819438	278	76574

CONCLUSION:

It was found in the observation that the copper based cluster shows a unique dissociation behavior. There was no loss of water molecule (-18) upon fragmentation of copper bound tripeptide. For all the others, there was loss of a water molecule. When comparing the ratio of intensities, it was observed that the Ni bound to tripeptide has more intensity of $-\text{CO}_2$, $-\text{H}_2$ than $-\text{H}_2\text{O}$. Whereas the others have greater intensity of $-\text{H}_2\text{O}$ than $-\text{CO}_2/-\text{CO}_2$, $-\text{H}_2$. The metals Cu, Mn and Zn combined with tripeptide lost CO_2 (44) upon fragmentation of the parent ion, Ni and Fe combined with tripeptide lost CO_2 and H_2 (46) upon fragmentation.

REFERENCES:

- [1] J.D. Carron, J.-W. Shin, *Chemical Physics Letters* 598 (2014) 102.