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Tandem Mass Spectrometric Analysis of Phosphoamino Acid-metal Ion Complexes

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Abstract for Research

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Tandem mass spectrometric analysis of phosphoamino acid-metal ion complexes

The complexes of phosphoamino acid-metal ions are prepared by mixing both of them and passing them through electrospray ionization and collision- induced dissociation to carry out tandem mass spectrometry. The fragmentation products of phosphoamino acid-metal ions generated are studied further. The dissociation patterns of each phosphoamino acid with metal ions are studied.

ABSTRACT:

The complex of phosphoamino acid with metal ions was prepared by mixing both solutions and analyzed using ESI MS/MS technique. In this study, O-phospho-L-serine and O-phospho-L-tyrosine were used as phosphoamino acids and single charged metal ions such as Li^+, Na^+, K^+, Rb^+, Cs^+ and Ag^+ were used to study the dissociation patterns when combined with phosphoamino acid. The complexes were successfully fragmented in mass spectra using ESI MS/MS technique and the fragmentation products were studied and the dissociation patterns were proposed.

INTRODUCTION:

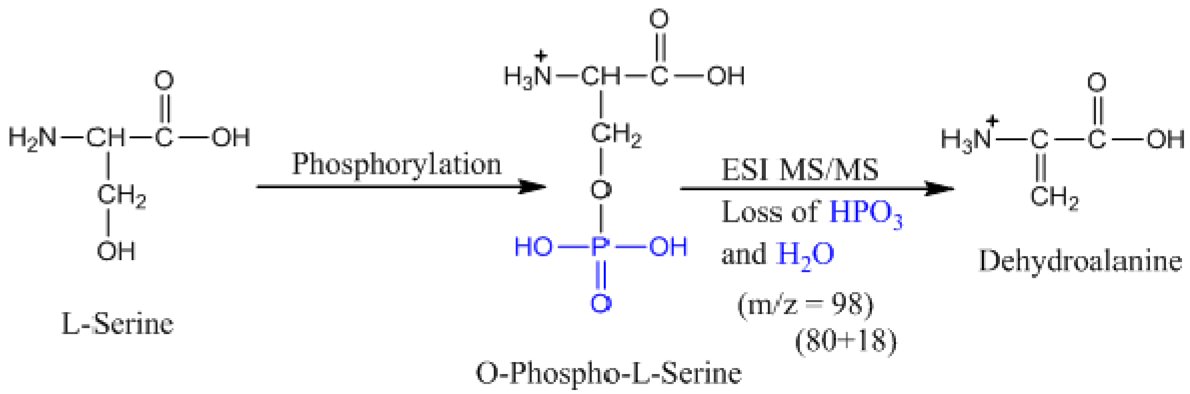
The biomolecules were initially analyzed using electrophoresis, chromatographic or ultracentrifugation methods for determination of its molecular weight. But the results were not very accurate because of structural conformation, Strokes radius and hydrophobicity which cause changes in structure in addition to molecular weight.

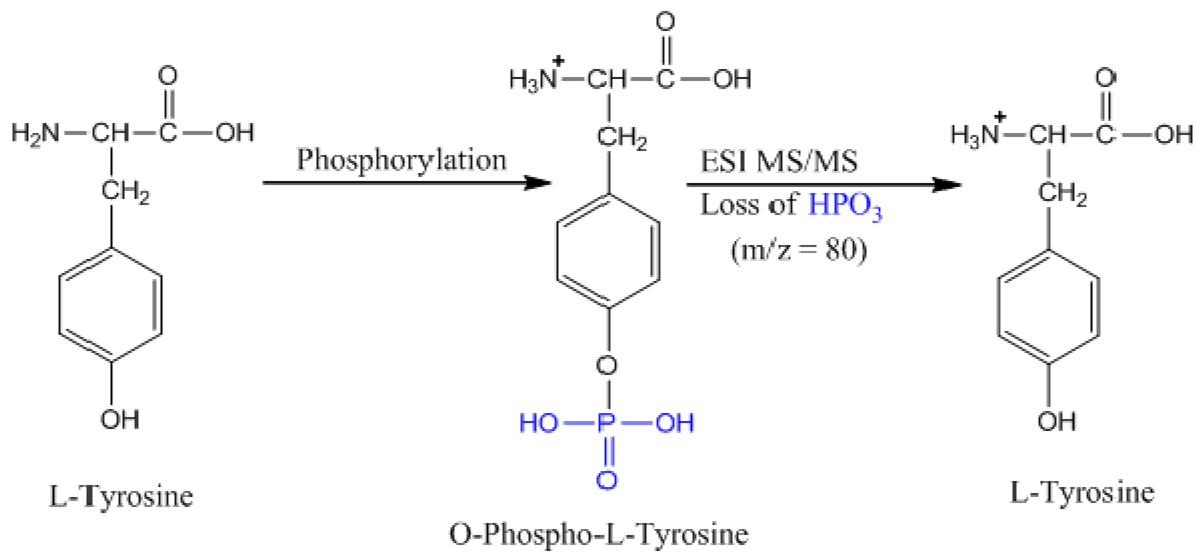
Electrospray ionization (ESI) is an ionization technique which is used to produce singly charged ions in mass spectrometry using proton or cation as charge carrier. It provides high resolution for analysis of biomolecules of high molecular weight.

The proteins synthesized from ribosomes undergo various post-translational modifications such as glycosylation, phosphorylation, sulfation, hydroxylation, carboxylation, formation of disulfide bonds and acetylation of N-terminal acid[1]. The phosphoamino acid as O-phospho-L-serine and O-phospho-L-tyrosine were studied. The phosphorylation is usually observed on "R" side chain group in serine, tyrosine and threonine amino acids. The phosphate group (H_2PO_3) is usually attached to oxygen atom of side chain of serine, tyrosine and threonine.

The fragmentation of phosphoamino acids shows the mass difference of 98 m/z which indicates loss of the phosphate (HPO_3) group and water (H_2O) molecule from amino acid such as serine and threonine. Moreover it shows the loss of 80 m/z which indicates loss of phosphate (HPO_3) group from tyrosine.

[1]Edmond de Hoffmann,VincentStroobant; *Mass Spectrometry Principles and Applications*, John Wiley and Sons, Ltd, 3rd edition, **2007**; 305-306.





EXPERIMENTAL CONDITIONS:

Instrumental conditions:

All the experiments were carried out using Agilent technologies MSD Trap XCT.

Nebulizer: 30 psi

Dry gas: 7.0 L/min

Dry temperature: 300 ˚C

Solvent:

500 mL of 1:1 % v/v solution of methanol: water was used as solvent for the experiment.

Sample Preparation:

Phosphoamino acids- O-phospho-L-serine and O-phospho-L-tyrosine

Metal salts: Lithium chloride, Sodium chloride, Potassium chloride, Rubidium chloride, Cesium chloride and Silver acetate.

PROCEDURE:

- 0.005 M solutions of phosphoamino acids were prepared using the above mentioned solvent. For dissolving O-phospho-L-tyrosine, a few drops of hydrochloric acid were added.
- 0.005 M solutions of metal salts were prepared using the above mentioned solvent. This will generate metal ions which will form complex with phosphoamino acids.
- 3 mL solution of each phosphoamino acid was mixed with 3 mL of each metal ion solution and the complex solution was prepared.
- For dissolving O-phospho-L-tyrosine, hydrochloric acid was used. When solution of Ag^+ was mixed with O-phospho-L-tyrosine, precipitation occurred because of formation of $AgCl_2$ and the resulting solution was not further studied for ESI MS/MS.
- About 500 µL of complex solution was transferred to ESI syringe.
- The syringe was placed in syringe pump and capillary tube was connected to ionization source of mass spectrometer.
- The MSn parameters were set in ChemStation software for manual isolation and fragmentation.
- The syringe pump was started (0.3 mL/hr) and mass spectrum was acquired.
- When the precursor ion intensity was stabilized, it was isolated and ion was fragmented by adjusting amplitude voltage. The amplitude voltage was different for all solutions according to the intensity of mass spectrum acquired.
- The MS results were saved and printed to propose the fragmentation pattern.

RESULTS AND DISCUSSION:

Solvent preparation:

400 mL of HPLC grade water and 100 mL of HPLC grade methanol was poured in measuring cylinder to produce 500 mL solution and the resulting solution was transferred to suitable container. The resulting solution was used for dissolving phosphoamino acids and metal ions.

Sample preparation:

0.005 M solution mixture of phosphoamino acids with metal ions were studied using ESI MS/ MS and following spectra was generated which was further studied to propose the fragmentation patterns.

ESI MS/MS of **O-phospho-L-serine** and **O-phospho-L-tyrosine**are as shown below:

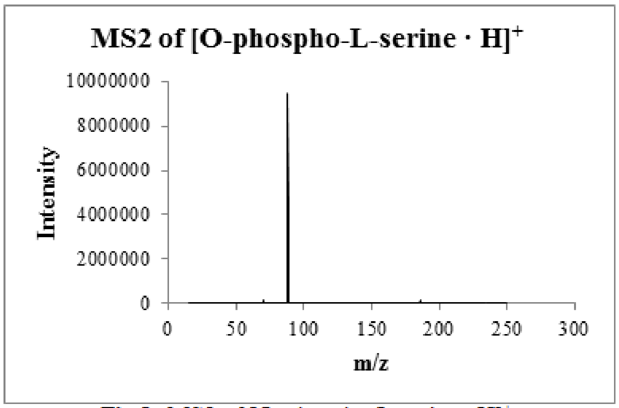


Fig 1: MS2 of [O-phospho-L-serine · H]$^+$

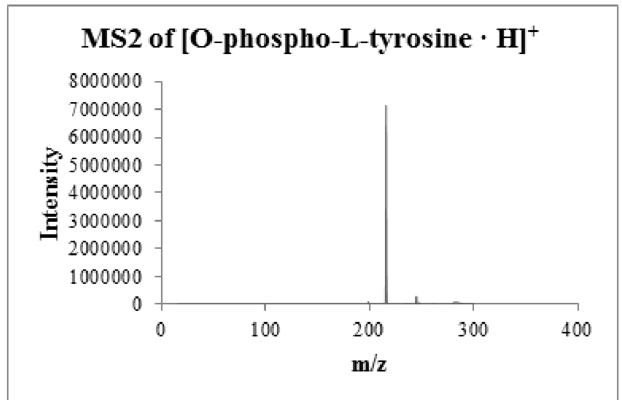


Fig 2: MS2 of [O-phospho-L-tyrosine · H]$^+$

From the above Figure 1 and 2, the following fragmentation pattern was proposed as shown in Table 1:

Table 1: Proposed fragmentation patterns of phosphoamino acids

Sr. No.	Phosphoamino acid	m/z ratio	Loss of m/z	Precursors
1.	O-phospho-L-serine	185.9	-	O-phospho-L-serine + H^+
		88.2	97.8	Loss of **HPO_3+ H_2O**
		70.4	17.8	Loss of H_2O
2.	O-phospho-L-tyrosine	262	-	O-phospho-L-tyrosine + H^+
		244.9	17.1	Loss of NH_3
		216	46	Loss of CO + H_2O or CO_2 + H_2
		199	17	Loss of NH_3

As shown in Table 1, the O-phospho-L-serine showed the peak indicating loss of 98 m/z as discussed in introduction.

But O-phospho-L-tyrosine did not showed the peak indicating loss of 80 m/z as discussed in introduction.

ESI MS/MS of **O-phospho-L-serine** with **metal ions**:

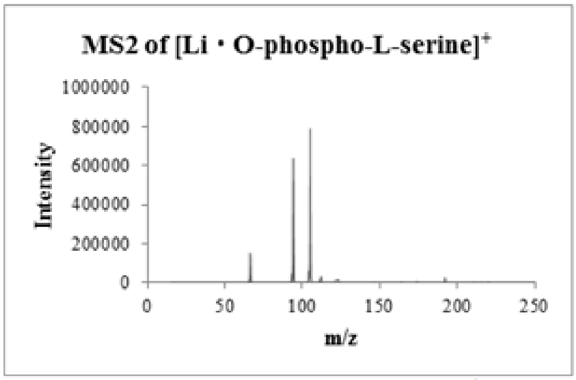


Fig 3: MS2 of [Li · O-Phospho-L-Serine]$^+$

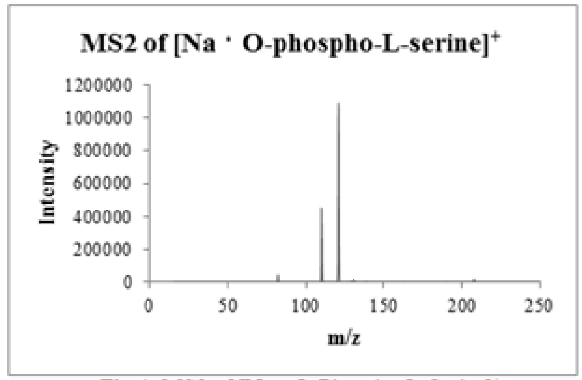


Fig 4: MS2 of [Na · O-Phospho-L-Serine]$^+$

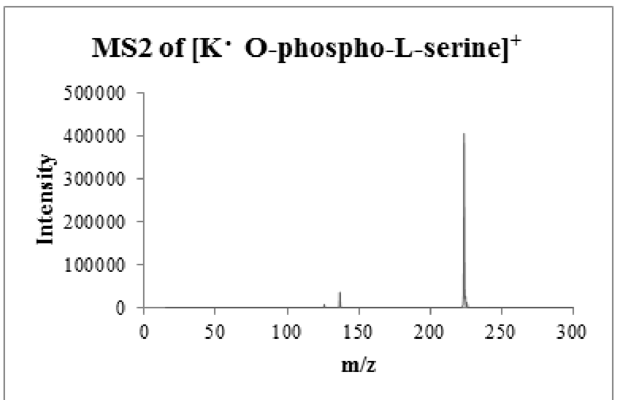


Fig 5: MS2 of [K · O-Phospho-L-Serine]$^+$

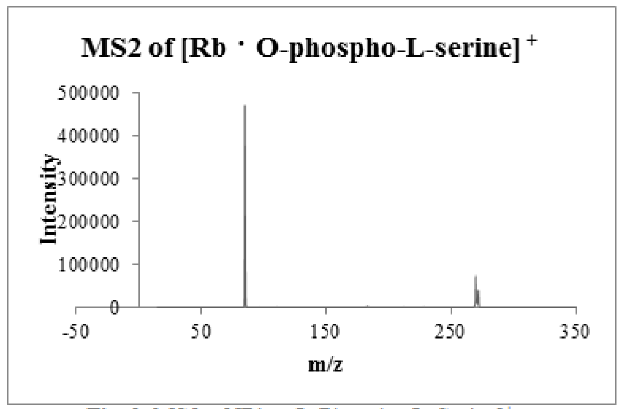


Fig 6: MS2 of [Rb · O-Phospho-L-Serine]$^+$

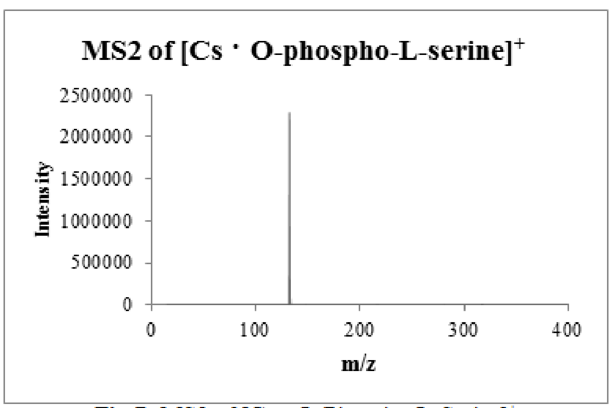


Fig 7: MS2 of [Cs · O-Phospho-L-Serine]$^+$

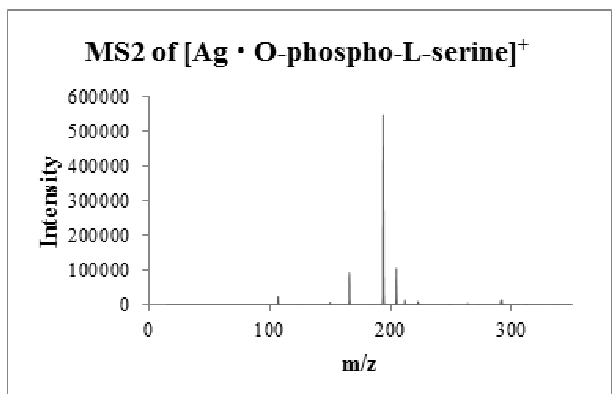


Fig 8: MS2 of [Ag · O-Phospho-L-Serine]$^+$

From the above Figure 3, 4, 5, 6, 7 and 8, the following fragmentation patterns are proposed for O-phospho-L-serine with metal ions as shown in Table 2:

Table 2: Proposed fragmentation patterns of O-phospho-L-serine with metal ions

Sr. No.	**O-phospho-L-serine + Metal ion**	**m/z ratio**	**Loss of m/z**	**Precursors**
1.	With Li^+	191.9	-	O-phospho-L-serine + Li^+
		112	79.9	Loss of **HPO_3**
		105	17.9	Loss of H_2O
		94.2	17.8	Loss of H_2O
		66.4	27.8	Loss of CO
2.	With Na^+	208	-	O-phospho-L-serine + Na^+
		128	80	Loss of **HPO_3**
		120.9	87.1	Loss of **Serine**
		110.1	97.9	Loss of **HPO_3+ H_2O**
		82.2	27.9	Loss of CO
3.	With K^+	224	-	O-phospho-L-serine + K^+
		136.9	87.1	Loss of **serine**
		126	98	Loss of **HPO_3+ H_2O**
4.	With Rb^+	269.8	-	O-phospho-L-serine + Rb^+
		182.8	87	Loss of **serine**
		85.1	184.9	Loss of **metal ion**
5.	With Cs^+	318	-	O-phospho-L-serine + Cs^+
		132.8	185.2	Loss of **metal ion**
6.	With Ag^+	292	-	O-phospho-L-serine + Ag+
		204.7	18.1	Loss of H_2O
		193.8	18.3	Loss of H_2O
		165.9	27.9	Loss of CO
		149.9	16	Loss of O
		106.9	86.9	Loss of **serine**

From the above Table 2, we can summarize the fragmentation pattern for loss of serine, phosphoserine or phosphate group according to an increase in atomic radii which may result in different fragmentation pattern as shown in below Table 3.

Table 3: Proposed effect of atomic radii of metal ionson fragmentation of O-phospho-L-serine

Loss of	Li^+	Na^+	K^+	Rb^+	Cs^+	Ag+
Phosphate (80)	**Yes**	**Yes**				
Serine (87)		**Yes**	**Yes**	**Yes**		**Yes**
Phosphate + H_2O (98=80+18)		**Yes**	**Yes**			
Metal ion (185)				**Yes**	**Yes**	

From the Table 3, we can say that when the atomic radius is small, it results in loss of phosphate group as the metal ions are tightly bounded to O-phospho-L-serine. But as the atomic radii increases, the metal ions are loosely bounded to O-phospho-L-serine and hence the fragmentation of metal ion takes place.

ESI MS/MS of **O-phospho-L-tyrosine** with **metal ions**:

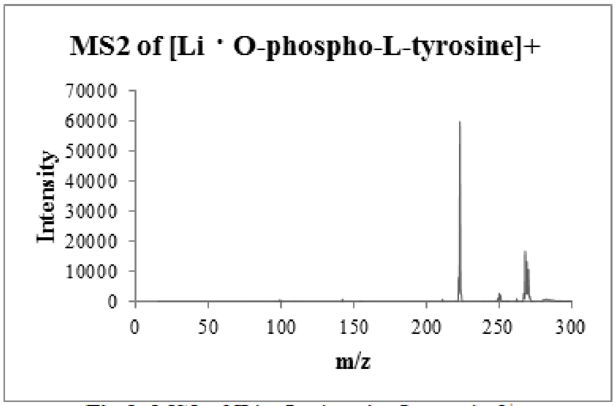


Fig 9: MS2 of [Li · O-phospho-L-tyrosine]$^+$

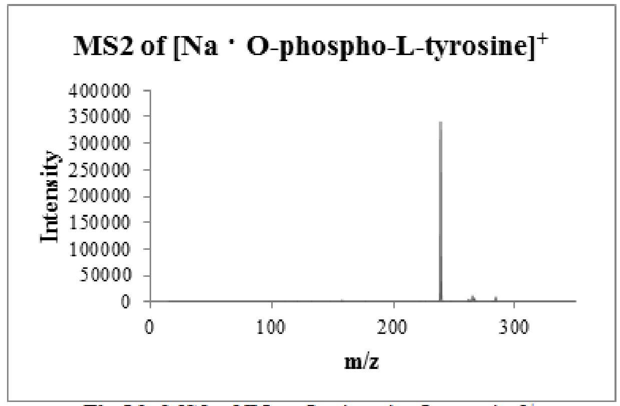


Fig 10: MS2 of [Na · O-phospho-L-tyrosine]$^+$

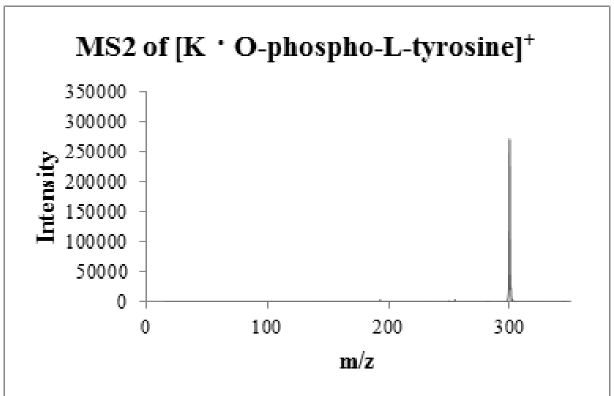


Fig 11: MS2 of [K · O-phospho-L-tyrosine]$^+$

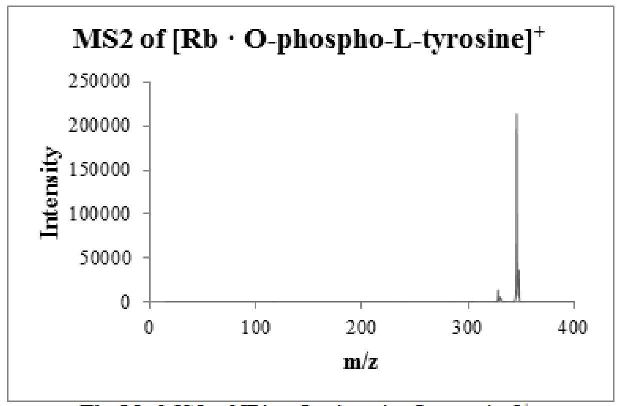


Fig 12: MS2 of [Rb · O-phospho-L-tyrosine]$^+$

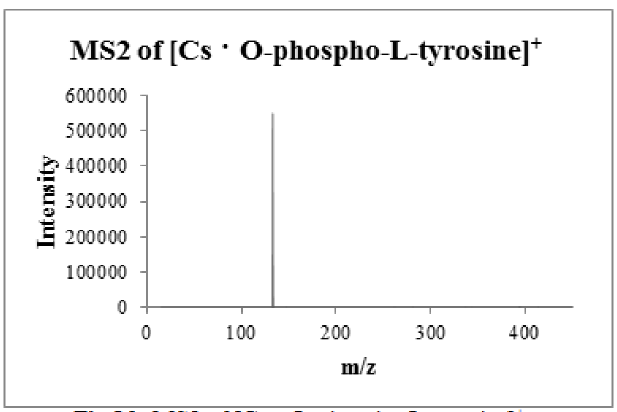


Fig 13: MS2 of [Cs · O-phospho-L-tyrosine]$^+$

From the above Figure 9, 10, 11, 12 and 13, the following fragmentation patterns are proposed for O-phospho-L-tyrosine with metal ions as shown in Table 4:

Table 4: Proposed fragmentation patterns of O-phospho-L-tyrosine with metal ions

Sr. No.	O-phospho-L-tyrosine + Metal ion	m/z ratio	Loss of m/z	Precursors
1.	With Li^+	268	-	O-phospho-L-tyrosine + Li^+
		250	18	Loss of H_2O
		223	45	Loss of CO_2 + Hor CO + OH
		211	57	Loss of glycine
		142.1	80.9	Loss of **HPO_3**
2.	With Na^+	284.4	-	O-phospho-L-tyrosine + Na^+
		238.9	45.5	Loss of CO + H_2O or CO_2 + H_2
		158	80.9	Loss of **HPO_3**
3.	With K^+	299.9	-	O-phospho-L-tyrosine + K^+
		254.9	45	Loss of CO_2 + H or CO + OH
4.	With Rb^+	345.9	-	O-phospho-L-tyrosine + Rb^+
		328.2	17.7	Loss of H_2O
5.	With Cs^+	394	-	O-phospho-L-tyrosine + Cs^+
		132.8	261.2	Loss of **metal ion**

From the above Table 4, we can summarize the fragmentation pattern for loss of phosphate or metal ion according to an increase in atomic radii which may result in different fragmentation pattern as shown in below Table 5.

Table 5: Proposed effect of atomic radii of metal ion on fragmentation of O-phospho-L-tyrosine

Loss of	Li^+	Na^+	K^+	Rb^+	Cs^+
Phosphate (80)	**Yes**	**Yes**			
Metal ion (261)					**Yes**

From the Table 5, we can say that when the atomic radius is small, it results in loss of phosphate group as the metal ions are tightly bounded to O-phospho-L-tyrosine. But as the atomic radii increases, the complex did not showed the loss of phosphate group or metal ions. Although the complex did show fragmentation of metal ion in case of Cs^+.

Summary and Conclusion:

ESI is a very useful technique in mass spectrometry to study singly charged ions as it is highly sensitive technique. The ESI MS/MS result gives meaningful information about the fragmentation products. It has been found to be very useful technique for studying biomolecules. Based on current results we can conclude that there was gradual loss of phosphate group to metal ions in case of O-phospho-L-serine. While no profound loss of phosphate group to metal ions was observed in case of O-phospho-L-tyrosine. The experiment showed better results of fragmentation pattern with O-phospho-L-serine as compared to that of O-phospho-L-tyrosine. This may be the result of improper experimental conditions for O-phospho-L-tyrosine.