

Unique Fragmentation Pathways Observed in Corona Discharge Electrochemical/Electrospray Ionization (CD ECI/ESI) MS

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Objective

To determine the mechanism of diagnostic fragmentation observed using Corona Discharge Electrochemical/Electrospray Ionization (CD ECI/ESI) on peptides without electrochemical label.

Introduction

The electrochemical aspects of electrospray ionization are well documented but until now, the electrochemistry native to electrospray has rarely been exploited using LC-MS conditions. One of the reasons may be that special hardware was required. However, electrochemical oxidation and reduction are the most sensitive detection methods. Therefore, we have explored whether we can create electrochemical/electrospray ionization (ECI/ESI) conditions using a standard AP/ESI ion source. Using corona discharge as an electron removal pathway, we have demonstrated in poster TP472 that efficient electrochemistry can be achieved using a standard ESI source. In this poster, we describe some additional observations we made using Corona Discharge ECI/ESI conditions where unlabeled peptides fragment in an unexpected but diagnostically useful manner. We propose a mechanism to explain the observed fragmentation.

Experimental Design

In this study a standard, commercial API/ESI ion source was mounted on a Waters LCT TOF mass spectrometer. Initially, using ferrocene labeled compounds we developed conditions to obtain ECI sensitivities equal to or greater than ESI. We determined one critical objective to be the maximization of the corona discharge present at the end of the ESI capillary. Capillary voltage, desolvation gas temperature and capillary position were all adjusted to increase the amount of observed corona discharge and subsequently the observed signal from the compounds. Another important design feature was to use a ground electrode behind the ESI tip to deflect the corona discharge plasma away from the ECI/ESI ion path eliminating unwanted ion-molecule reactions and neutralization of the CD ECI/ESI generated ions. Using these optimized CD ECI/ESI conditions and unlabeled glycopeptide and phosphopeptide standards, we were able to induce diagnostically useful fragmentation similar to CID in a single TOF instrument.

Results: Glycopeptides

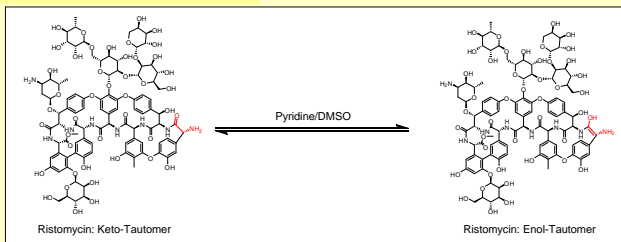


Figure 1. Tautomerization Reaction of Ristomycin in Pyridine/DMSO.

Glycopeptides

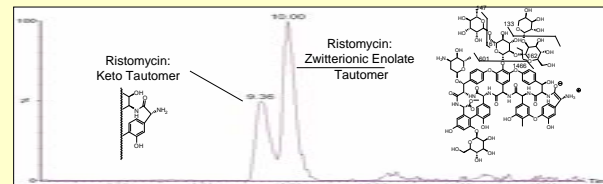


Figure 2. CD ECI/ESI LC/MS of Ristomycin and its zwitterionic enolate isomer (inset shows enolate form with characteristic fragments (see also Fig. 4).

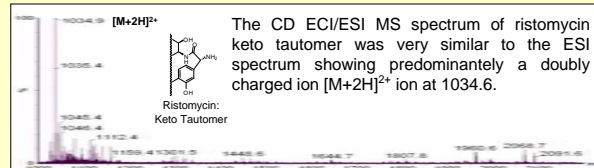


Figure 3. CD ECI/ESI Mass Spectrum of Ristomycin

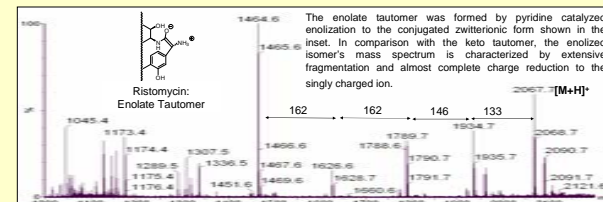


Figure 4. CD ECI/ESI Mass Spectrum of the Enolate Isomer of Ristomycin with characteristic fragments; Note the CD ECI/ESI fragmentation is very similar to that generated by CID of the doubly charged ion of ristomycin (not shown).

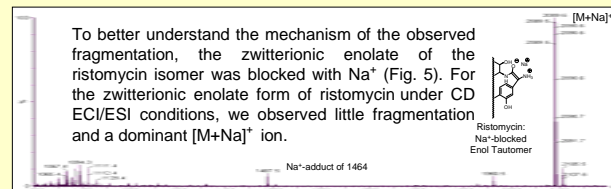


Figure 5. CD ECI/ESI Mass Spectrum of Sodium Blocked Enolate Isomer of Ristomycin

Acknowledgements

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Phosphopeptides

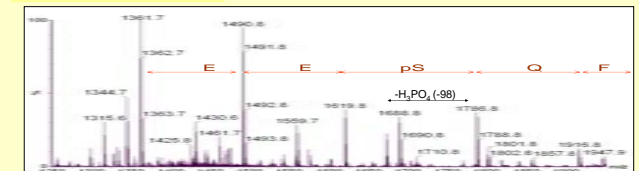


Figure 6. CD ECI/ESI Mass Spectrum (partial) of Phosphopeptide FQpSEEQQQTEDELQDK.

Figure 6 shows RP-HPLC CD ECI/ESI providing diagnostic fragmentation of the phosphopeptide FQpSEEQQQTEDELQDK. The location of the phosphorylation site on the serine is quite easily deduced from this series. Under CD ECI/ESI conditions, a loss of phosphoric acid (-98) is also observed.

Suggested Mechanism



Figure 7. Corona Discharge observed in ECI/ESI LC/MS conditions

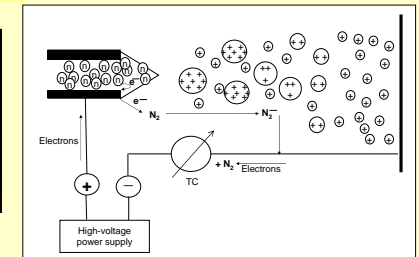


Figure 8. Overview ECI/ESI mechanism

We propose that under ECI/ESI conditions, the observed corona discharge (Fig. 7) constantly removes electrons from the analyte, thus ionizing the analyte (Fig. 8). Fragmentation occurs when an ionized group chelates with transition metals (such as Fe) from the stainless steel capillary. This is the case in the enolate form of ristomycin as well as in the phosphopeptide FQpSEEQQQTEDELQDK but not in the keto form of ristomycin. When Na⁺ prevented other metal ions present in the stainless steel capillary from chelating with the zwitterionic enolate moiety, no fragmentation was observed. We refer to this type of fragmentation as electrochemically initiated metal catalyzed fragmentation.

Conclusions

Under CD ECI/ESI conditions we have been able to fragment unlabeled peptides in a diagnostic manner similar but not identical to that obtained from collisional induced fragmentation experiments.

In contrast to CID, where neutral loss of phosphate often occurs before the fragmentation of the peptide chain, under CD ECI/ESI, the position of the phosphorylation site could easily be determined. The mechanism for this fragmentation appears to involve electrochemical initiation along with metal chelation of the peptide. The metals present in the stainless steel capillary bind to active chelating groups on some peptides.

The advantages of this fragmentation technique are:

1. It only requires minimal hardware.
2. The entire fragmentation pattern is available in one spectrum, no MSⁿ required.
3. The sensitivity is comparable to basic ESI sensitivity.
4. Novel diagnostic fragmentation can be readily created by simply changing the capillary material.