

# The Effect of Fixed Charge Modifications on Electron Capture Dissociation

Xiaojuan Li<sup>1</sup>, Jason J. Cournoyer<sup>1</sup>, Cheng Lin<sup>1</sup>, and Peter B. O'Connor<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118

## Introduction

Electron capture dissociation (ECD) is the dissociative recombination of multiply charged polypeptide molecules with low energy electrons.<sup>[1-2]</sup> To test the hypothesis that H<sup>·</sup> radical is required for the N-C<sub>α</sub> cleavage<sup>[3]</sup>, the ECD spectra of two model peptides with one or two fixed charge tags respectively were obtained. The fixed charge tags functioned as stable radical traps that did not generate H<sup>·</sup> and inhibited, but did not eliminate, the typical c/z cleavages of the peptide backbone. The types of fragment ions were found to correlate with the number and location of the fixed charge tags on the peptides.

## Methods

Two model peptides used in this experiment were Amyloid β-protein peptides GSNKGAIIGLM (25-35) and FAEDVGSNKG (20-29). Each peptide was dissolved in potassium carbonate buffer (pH 9) containing ~0.05M 2,4,6-trimethylpyridinium tetrafluoroborate and purged with nitrogen gas.<sup>[4]</sup> The final concentration of the peptides was about 0.4 mM. The reaction was allowed to proceed for 18-24 hrs at room temperature, which converted the Lysine side chain and/or the N-terminal amino group of the peptide to charged 2,4,6-trimethyl pyridinium groups. The peptide derivatives were then purified by POROS 50 and each fraction of the eluate was analyzed by using a custom qQq-FT-ICR-MS (Fourier transform ion cyclotron resonance mass spectrometer) equipped with a nano-spray source.<sup>[5]</sup> Unlabeled, singly-labeled, and doubly-labeled model peptides were analyzed by ECD at ~10<sup>-5</sup> M in 50:50 methanol: water with 1% formic acid. Ions were accumulated for 100-600 milliseconds in the front end quadrupole before entering the cell where they were irradiated with low energy electrons.

## Results

The singly tagged peptides generated fewer backbone cleavages than peptides without a fixed charge tag. The untagged Amyloid β-protein peptide (25-35) generated both b and c ions, while the peptide with one fixed charge located near its N-terminus had c ions as the most abundant ions. As previously observed<sup>[6]</sup>, the abundance of these c ions decreased and side chain cleavages increased with one fixed charge when compared to the fragment ions without a fixed charge.

The ECD of Amyloid β-protein peptide (20-29) generated c, z<sup>·</sup> and y ions. The abundance of c ions decreased and side chain cleavages increased with one fixed charge. Because the fixed charge is located near the C-terminus, this modified peptide generated both c and z<sup>·</sup> ions. The data indicated the N-terminal amine group and Lys side chain ε-amino group are the preferential neutralization sites, as only fragment ions containing the fixed charge tag could be detected. Thus, the tag position dictated what type of ions was generated.

For doubly charged peptides, both peptides showed fewer backbone cleavages and more side chain cleavages than their singly tagged counterparts. Amyloid β-protein peptide (20-29) with a fixed charge located at each end of this peptide (Fig. 1) had more backbone cleavages than Amyloid β-protein (25-35) with both fixed charges located near its N-terminus. This may be because the radical on the tag has a preference to abstract an H<sup>·</sup> from its vicinity. Separated radical positions would have a higher likelihood of generating backbone fragments after H<sup>·</sup> abstraction because of higher conformational heterogeneity.

The tag method was used to specifically add fixed charges at Lysine ε-amino group<sup>[4]</sup>. In the case of doubly tagged peptides here with sing Lys residue, the fixed charges were located at the N-terminus and the Lysine ε-amino groups. It means this method may also add one fixed charge group at the N-terminus of certain target peptides. So by using this method, peptides with one or more fixed charges may be generated depending on the number of Lysine residues and the nature of peptides.

## Conclusion

The fixed charge tag after neutralization will not generate  $H\cdot$  to facilitate the ECD process, but it can abstract an  $H\cdot$  radical from its nearby neighbors to open new pathways for the backbone and side chain cleavages. These data agree with that the  $H\cdot$  radical generation is important for c and z $\cdot$  ion production. However, several c and z $\cdot$  ions were observed in the doubly tagged peptides which means that an  $H\cdot$  radical is not always required for c/z cleavage which can also be induced by other radicals, albeit less frequently. Additionally, several internal fragments were observed in the spectra. These data support the ECD free radical cascade mechanism.

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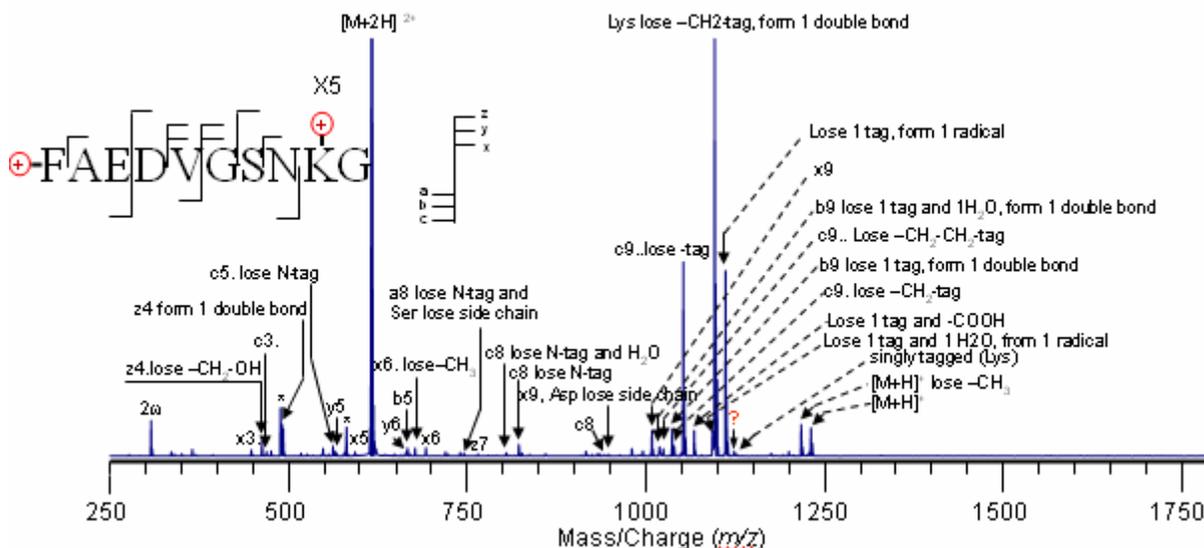


Fig.1 ECD spectrum of Amyloid  $\beta$  protein peptide 20-29 doubly tagged