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DISSOCIATION STUDY OF A CHEMOTACTIC PEPTIDE N-FORMYL-MET-LEU-PHE

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OVERVIEW-

N-formyl-Met-Leu-Phe (fMLF) is the smallest chemotactic peptide that can induce all major phagocyte functions, and it is one of the most potent chemoattractants for phagocytic leukocytes. Even though it has been studied via other analytical methods such as X-ray diffraction and NMR, there are no mass spectrometry data on the fragmentation study of this peptide. This research focuses on the dissociation mechanism of fMLF in the gas phase by employing mobile proton model. The result from this work is also helpful for the interpretation of the dissociation of other N-formylmethionyl peptides, which are known to be chemoattractants for neutrophils and macrophages.

INTRODUCTION –

It has long been observed that viable bacteria in infected tissues attract polymorphonuclear leucocytes. In 1984, Marasco and coworkers[1] identified N-formyl-Met-Leu-Phe as the major peptide with chemotactic activity. X-ray crystallographic study indicated that the interaction between N-formyl-Met-Leu-Phe and immunoglobulin light chain involved a formation of a hydrogen bond at the N-formyl group.[2] The methionine side chain usually occupies a hydrophobic pocket in the receptor.

Mobile proton model[3] was established based on the work from many research groups. Surface-induced dissociation (SID) and other techniques were used to validate this model. The activation energy in MS/MS experiments is considered to be the driving force for the proton to move from the basic side-chain to the peptide backbone to induce dissociation, and the energy required to move the proton depends on the amino acid composition.

Methionine and methionine containing peptides have been studied via tandem mass spectrometry methods. Communication from Harrison's group showed that the b_1 ion derived from methionine is a stable species.[4] O'Hair and coworkers[5] presented that the charge-directed neighboring group mechanism dominates the dissociation of methionine modifications.

METHODS-

Waters® Q-Tof™ Mass Spectrometer

Source: ESI+
Capillary (kV): 3.3
Cone (V): 30
Collision Gas: Argon
Collision Energy (eV): 20

Infusion:

Flow Rate (uL/min): 20
Solvent: 50% ACN/50% H_2O, 0.2% Formic Acid

RESULTS AND DISCUSSION-

Protonated N-formyl-Met-Leu-Phe can readily be formed under electrospray conditions. But the protonation site for this peptide is hard to determine due to the fact that Met, Leu, and Phe are neutral amino acids and the formyl group is at the N-terminus. In the collisionally activated dissociation (CAD) spectrum of protonated fMLF, immonium ions from methionine, leucine and phenylalanine are found together with b_3, a_3, b_2, a_2 and y_1 ions ***(Figure 1)***. This indicates that after the fragmentation of the protonated fMLF molecular ion, charge can be relocated to any of the nitrogens in the peptide backbone. Due to the fact that there is no basic site in fMLF, it is rational to assume that the proton from the protonated fMLF precursor ion is shared by all the amino acids instead of being occupied by a single amino acid. The most probable structure for

protonated fMLF is a mobile proton model with the proton being solvated by the carbonyl oxygens of the peptide backbone. This structure facilitates the backbone cleavages during peptide fragmentation, which gives different a, b, y, and immonium ions with the charge on different places. CAD results from other N-formylmethionyl peptides such as N-formyl-Met-Phe, N-formyl-Met-Met-Met, and N-formyl-Met-Phe-Met show similar patterns as N-formyl-Met-Leu-Phe, and the fragmentation mechanism can also be interpreted by this mobile proton model.

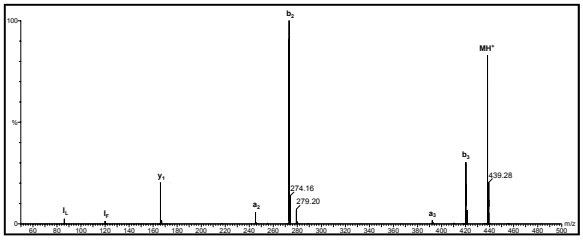


Figure 1. Low Energy CAD of fMLF

B type ions are the dominant fragment ions in low energy CAD spectra of protonated peptides and these ions are known to have protonated oxazolone structures.[6] But b_1 ions are usually not observed in peptide fragmentation because there is no carbonyl group available at the N-terminus of unmodified peptides. In the low energy CAD spectra of protonated N-formyl-Met-Phe-Met and N-formyl-Met-Met-Met ***(Figure 2)***, even though with low intensities, b_1 ions are clearly observed. Similarly, the b_1 ion is also a detectable fragment in the low energy CAD spectrum of protonated N-formyl-Met-Phe. The structure of b_1 ions can easily be explained by the formation of protonated oxazolone by including the carbonyl group at N-terminus.

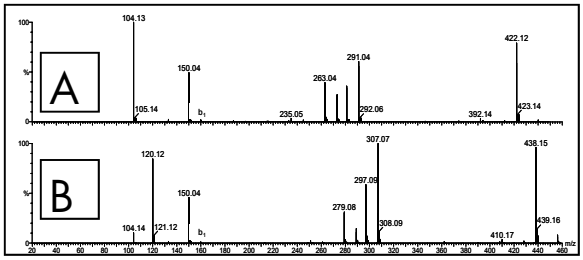


Figure 2. Low energy CAD spectra of N-formyl-Met-Met-Met (A) and N-formyl-Met-Phe-Met (B)

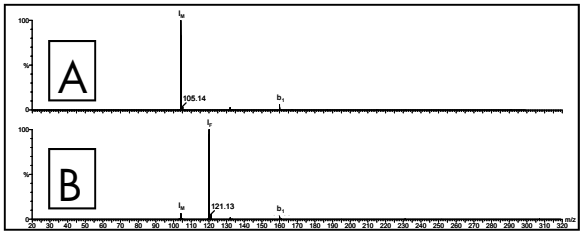
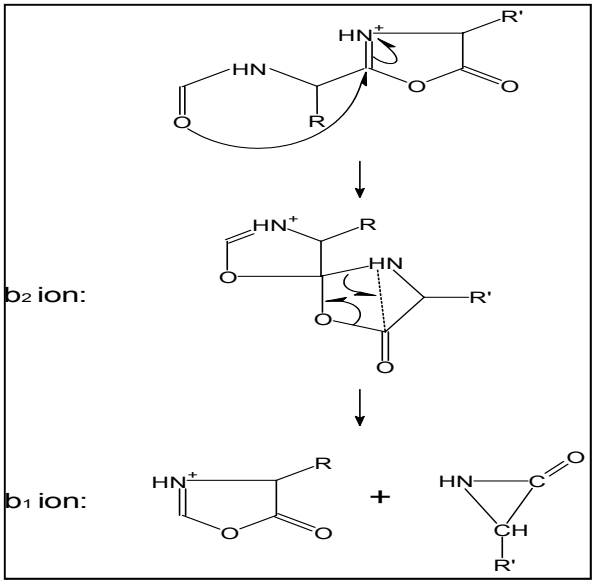


Figure 3. Low energy CAD of b2 ions from fMMM (A) and fMFM (B)



Scheme 1. Mechanism for the formation of b_1 ion from b_2 ionR: Met side chain; R′: Met or Phe side chain

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Low energy CAD spectra of b_2 ions from N-formyl-Met-Phe-Met and N-formyl-Met-Met-Met are shown in ***(Figure 3)***. Immonium and b_1 ions are the main daughter ions found in the CAD experiments. The fragmentation mechanism of b_2 ion from N-formyl-Met-X-Met (X = Met or Phe) is demonstrated in ***(Scheme 1)***.
The structure of the b_1 ion can be reconciled with a protonated oxazolone structure with nucleophilic attack of the carbonyl oxygen from the formyl group at the N-terminus to the oxazolone ring at the electrophilic immonium carbon. The coproduced neutral fragments in this process can also be HN=CHR' plus CO.

CONCLUSIONS-

- The protonation of N-formyl-Met-Leu-Phe and other N-formyl-methionyl peptides can be explained by the mobile proton model with the mobile proton being solvated by carbonyl oxygens in the peptide backbone.
- The structure of the b_1 ion from N-formyl peptides has a protonated oxazolone structure and it can be readily formed from the b_2 ion by nucleophilic attack of the carbonyl oxygen from the formyl group at the N-terminus to the oxazolone ring at the electrophilic immonium carbon.

REFERENCES–

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