

Comparing the Collision-Induced Dissociation of Trimethyl Lysine⁺ and Acetyl Lysine-H⁺

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Abstract

Experimental and computational results are reported on the reaction dynamics taking place within the collision-induced dissociation (CID) of $N_{\epsilon},N_{\epsilon},N_{\epsilon}$ -Trimethyl-L-lysine⁺ (TMe-lysine⁺) and N_{ϵ} -acetyl-L-lysine-H⁺ (acetyl-lysine-H⁺). These common post-translational modifications result in a mass difference of only 0.036 Dalton. Resolving these two species directly requires a high-resolution instrument and they share several CID peaks. Nevertheless, the mechanisms observed are strikingly different, particularly at lower internal energies. Reactivity is higher for acetyl-lysine-H⁺, with decomposition initiated via losses at the N- and C-termini. In contrast, unmodified lysine and TMe-lysine⁺ exhibit mechanisms that involve an initial loss of the PTM.

Introduction

Post-translational modifications (PTMs) of amino acids are common and result in an expanded functionality of proteins.¹⁻³ Methylation and acetylation of lysine are frequently observed within histone proteins. Tri-methylation and acetylation of a lysine at the side chain results in a mass difference of only ~ 0.036 Da, which requires high-resolution mass spectrometers to distinguish. However, the nature of these two species is considerably different. Tri-methylation of lysine by its very nature results in a charged species, while acetylation of the lysine side-chain results in no charge present. This fundamental difference results in significant changes in the reaction dynamics that take place during collision-induced dissociation (CID). The study of PTMs via tandem mass spectrometry (MS2) and CID is a common task both experimentally⁴⁻⁷ and computationally.⁸⁻¹³ In our prior work, we exam-

ined mono-, di, and tri-methylation of lysine using both techniques.¹⁴ In this work, we will directly examine the differences between $N_{\epsilon}, N_{\epsilon}, N_{\epsilon}$ -Trimethyl-L-lysine (TMe-lysine⁺) and N_{ϵ} -acetyl-L-lysine-H⁺ (acetyl-lysine-H⁺) using both experimental and computational means. Computationally, we employ direct dynamics simulations^{11,12} to sample the chemical reactivity of both TMe-lysine⁺ and acetyl-lysine-H⁺, and reveal atomic level information regarding the mechanisms of CID in each system. As in our previous study, we will analyze the direct dynamics simulations by employing graph theory, which has recently become a popular means of gaining deeper insight into such results.^{9,10,14–19} The use of graph theory allows for a straight-forward analysis of the isomers present for a given peak in a theoretical mass spectrum, as was illustrated in our previous work on methylated lysine.¹⁴

An outline for the remainder of the paper is as follows: in Section , we provide an overview of our computational and experimental methods; in Section , we present our results and compare the CID pathways between TMe-lysine⁺ and acetyl-lysine-H⁺; and in Section , we provide a summary.

Methods

Computational Approach

The techniques to simulate CID of MS2 systems are well documented in the literature.^{8,11,20} Moreover, this work uses the results for TMe-lysine⁺ that were initially calculated by some of us in Lucas *et al*¹⁴ along with new simulations for acetyl-lysine-H⁺. Below, we will briefly describe the approach and refer the interested reader to the literature for greater details.

Structures and Simulation Method

Initial structures for both TMe-lysine⁺ and acetyl-lysine-H⁺ were generated using Avogadro.²¹ TMe-lysine⁺ naturally has a +1 charge located at the side-chain PTM, while an excess proton was placed on the N-terminus of acetyl-lysine-H⁺. A simulated anneal approach using GROAMCS²² and the Gromas 54A7²³ potential modified using

the Automatic Topology Builder^{24–26} was implemented to obtain candidate low-energy structures through six heat-cool cycles in which the temperature was ramped to 1000 K over 100 ps, held at 1000 K for 100 ps and then cooled to 0 K over 100 ps. The resulting 0 K structures were optimized at the RM1 semi-empirical method²⁷ level as implemented in Mopac2016.²⁸ RM1 has produced good results for many previous MS2 simulations of protonated peptides.^{13,14,20,29–31} The structure with the lowest energy for each TMe-lysine⁺ and acetyl-lysine-H⁺ were selected as the starting point for our direct dynamics simulations.

In our previous work involving TMe-lysine⁺,¹⁴ we used a microcanonical sampling scheme to impart a statistical distribution of internal energy,³² which produced results that are in good agreement with experiment. In that work, the most reactivity occurred for TMe-lysine⁺ with an internal energy of 400 kcal/mol. In this work, we will use these 400 kcal/mol trajectories as our initial data set for TMe-lysine⁺. For acetyl-lysine-H⁺, we considered internal energies of 250 and 300 kcal/mol. For each internal energy considered, 400 trajectories were calculated. The selected range of internal energies allowed for sufficient reactivity to explore the chemical space without producing large populations of low-mass products. As will be described in the results section, additional simulations were performed to sample rare events. Trajectories were numerically integrated using Hamilton's equation of motion along with a 6th order symplectic integration scheme,³³ for a maximum simulation time of 50 ps with a 1 fs step size and output written every 50 fs using our in-house simulation package tightly coupled with Mopac2012.³⁴ Given our focus is on charged fragments, neutral fragments were removed from the simulation when they were at least 15 Å away from a charge center. Trajectories were halted if the final charged fragment had an $m/z \leq 60$. All trajectories had excellent conservation of energy.

Simulated mass spectra were obtained using the same methods our group has described previously.^{9,35} Briefly, our in-house simulation code calculates and reports a connectivity matrix every 50 fs based on a bond order matrix that is averaged over a 5 fs window. In post analysis, these matrices are used to determine when connectivity changes,

such as fragmentation events and proton transfers. Combining this information with a graph theory analysis^{17,18} of final products allows for the automatic determination of structures associated with a particular m/z peak.

Experimental Approach

High purity samples of $N_\epsilon, N_\epsilon, N_\epsilon$ -Trimethyl-L-lysine and N_ϵ -acetyl-L-lysine, acetonitrile were obtained from Sigma Aldrich and used without further purification. All samples were prepared in a mass spectrometry grade 1:1 mixture of acetonitrile and water. High-resolution mass spectrometry data was acquired on a Bruker Maxis Impact HD (Quadrupole-Time of Flight) spectrometer using sodium formate solution as the mass calibrant and the standard heated electrospray ionization (ESI) source. Pseudo MS3 experiments were performed utilizing in-source fragmentation. Typical collision energies considered were 5, 10, 15, 20, 25, 30, and 35 eV using nitrogen within the CID cell.

Results and Discussion

Overview and general comparison between acetyl-lysine- H^+ and TMe-lysine $^+$

While the nominal mass of both acetyl-lysine- H^+ and TMe-lysine $^+$ are identical and the experimental measurements show that the two systems share several decomposition peaks, their reactivity is in fact strikingly different. An overview of the experimental results is provided in Figure 1 which shows an example MS2 spectra at a collision energy of 20 eV along with the experimental break-down curves. One immediate observation apparent from the experimental data regarding the amount of parent ion remaining is that acetyl-lysine- H^+ is significantly more reactive than TMe-lysine $^+$. This is also seen in the direct dynamics simulations. It should be noted once again that TMe-lysine $^+$ has no excess proton and that the charge arises from the nature of the side-chain modification. This lack of an excess proton is likely the primary reason for its reduced re-

activity. While at lower collision energies, there are significant differences in the experimental results, it is seen that at large collision energies, both species result in the m/z 84 peak preferentially.

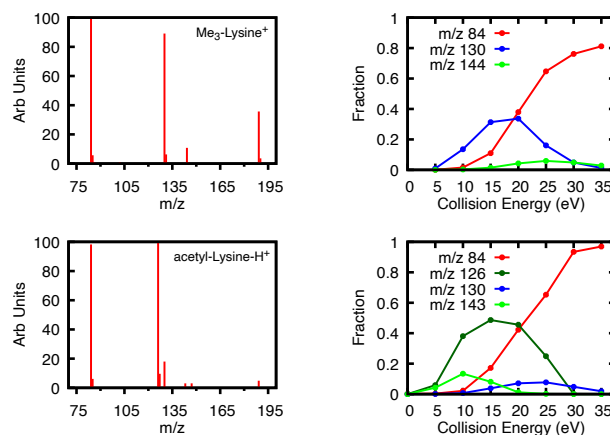


Figure 1: The experimental MS2 spectra for TMe-lysine $^+$ and acetyl-lysine- H^+ for a collision energy of 20 eV in the first column with experimental break-down curves provided in column 2. In the first column, the spectrum intensity is scaled to the most intense peak for each given spectrum. In the second column, the experimental break-down curves are relative to the total ion signal collected. The greater diversity in MS2 decomposition peaks for acetyl-lysine- H^+ is apparent.

Our simulations provided additional information and we start our computational analysis by presenting a comparison of the relative intensity of the most common peaks observed in our simulations for each system in Table 1. While acetyl-lysine- H^+ is more reactive, it is noteworthy that the majority of that reactivity occurs away from the PTM and involves the N- and C-terminus. This is a striking observation as the majority of the reactivity in TMe-lysine $^+$ is initiated at the side-chain. Hence, it can be concluded that the acetylation has hindered reactivity at the side chain in contrast to trimethylation, which retains more reactivity, primarily through the formation of trimethyl amine. While m/z 123 is a common experimental peak, it is not commonly seen in simulations, which will be discussed more below.

Table 1: A comparison of the major peaks observed in the simulated mass spectrum of acetyl-lysine- H^+ at 300 kcal/mol and TMe-lysine $^+$ at 300 and 400 kcal/mol reported as a fraction of all calculated trajectories.

m/z	acetyl-lysine- H^+ 300 kcal/mol	TMe-lysine $^+$ 300 kcal/mol	TMe-lysine $^+$ 400 kcal/mol
130 ^a	0.01	0.01	0.103
84 ^{a,b}	0.005	0.01	0.22
143 ^b	0.315	0	0.015
189 ^c	0.611	0.968	0.483

^a Loss includes a portion of the side-chain.

^b Loss of either $\text{C}(\text{OH})_2$ or $\text{H}_2\text{O} + \text{CO}$.

^c No fragmentation occurred.

Reactive pathways for acetyl-lysine- H^+

Our experimental measurements find that at moderate collision energies the primary CID product of acetyl-lysine- H^+ (m/z 189 - $[\text{H}_{17}\text{C}_8\text{N}_2\text{O}_3]^+$) is m/z 126 - $[\text{H}_{12}\text{C}_7\text{NO}]^+$, which corresponds to the loss of $\text{H}_2\text{O} + \text{CO} + \text{NH}_3$ - i.e. the loss of the N- and C-terminus leaving the side-chain intact. The m/z 126 peak has previously been identified as a novel marker ion for acetylated lysine by Kim *et al.*³⁶ At larger collision energies, a more significant population of m/z 84 ($[\text{H}_{10}\text{C}_5\text{N}]^+$) forms, which, as will be described below, can result from a secondary fragmentation of the m/z 126 decomposition product.

Our simulations find that the primary CID pathway for acetyl-lysine- H^+ involves the loss of $\text{C}(\text{OH})_2$ (or $\text{H}_2\text{O} + \text{CO}$) from the C-terminus to yield m/z 143 ($[\text{H}_{15}\text{C}_7\text{N}_2\text{O}]^+$). This chemical transformation occurs via a proton migration from the protonated N-terminus to the C-terminus. While the majority of the time this is a direct transfer, it is also possible for multiple proton hops to occur, with the acetyl's oxygen acting an intermediate protonation site. In either case, the most common final structure is protonated N-(5-iminopentyl) acetamide, making up 71.4% of the peak. Variations on this structure are seen in which a proton and/or the double bond has migrated, but all structures are straight-chain and maintain the same heavy atom sigma bonding framework, as shown in Figure 2. All other peaks within the simulation are minor pathways, and hence, we con-

clude that within the timescale of the simulations, side-chain loss has been largely deactivated.

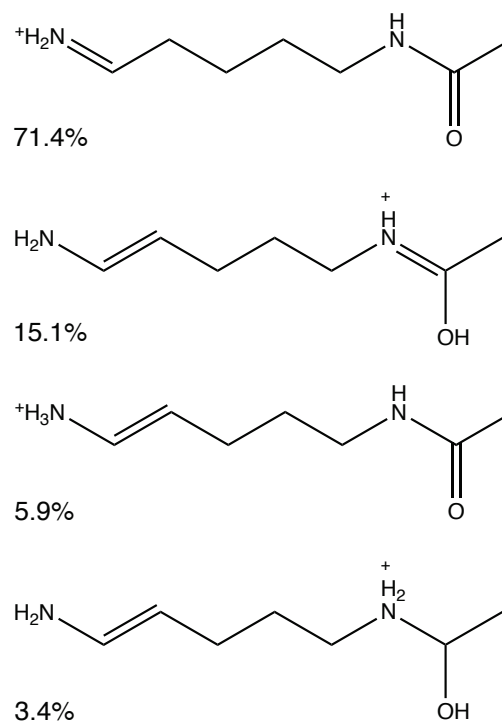


Figure 2: In simulations, the most commonly occurring structure in the m/z 143 peak is protonated N-(5-iminopentyl) acetamide at 71.4%. Several other structures in which a proton/double bond has migrated are also shown.

Within the initial set of trajectories, the primary experimental peak (m/z 126) was observed as a rare event in which m/z 143 was identified as an intermediate. To obtain a larger sample size of m/z 126 within simulations, two additional sets of calculations were performed: 1) 100 ps simulations of acetyl-lysine- H^+ and 2) simulations starting from the most common structure within the m/z 143 peak with a 100 ps simulation and an internal energy of 250 kcal/mol. While both approaches did result in larger m/z 126 populations, it was determined that the second approach would enable obtaining a sufficient sample of post m/z 143 reactivity with less computational expense.

It was found that m/z 143 primarily decomposes into m/z 126, 101 ($[\text{H}_{13}\text{C}_5\text{N}_2]^+$), 84, 56 ($[\text{H}_6\text{C}_3\text{N}]^+$), and 43 ($[\text{CH}_3\text{CO}]^+$). The order of frequency of these secondary decomposition products is m/z 101, 84, 43, 126, and 56, respec-

tively. As described above, m/z 126 and 84 are seen experimentally. Starting from acetyl-lysine- H^+ , the m/z 126 peak is formed via loss from both the N- and C-terminus, namely loss of $NH_3 + C(OH)_2/H_2O + CO$. In our simulations this most commonly occurs via a ring closure mechanism that is similar to pathway 4c of our previous work on lysine- H^+ .¹⁴ The m/z 84 peak forms through two different primary means: 1) directly from m/z 143 via the neutral loss of acetamide, and 2) indirectly via the subsequent loss from m/z 126 of the acetylation PTM as neutral ketene (CH_2CO). These mechanisms are shown in Figure 3. In our simulations, the direct loss pathway occurs $\sim 40\%$. In each case, the most common charged structure is N-protonated 1-Piperidine, which makes up a 0.59 fraction of the peak with an additional 0.29 of the peak composed of straight-chain structures that could interconvert to N-protonated 1-Piperidine given sufficient time and energy. The remaining structures are likely transient intermediates that are captured at the end of the simulation time window.

The m/z 101 and 43 peaks are also closely related to these primary decomposition products with m/z 101 corresponding to the loss of ketene (CH_2CO) from m/z 143, while m/z 43 is protonated ketene ($[CH_3CO]^+$). The former is likely a transient intermediate that will ultimately result in m/z 84, while the later could either transfer a proton back resulting in m/z 101 or could be the final product. The m/z 56 peak is the least commonly observed and forms following a hydrogen abstraction by the side-chain nitrogen from N-terminus from the β carbon of the side-chain to produce protonated aminoallene. Experimentally, our instrument cannot observe either m/z 43 or 56.

Reactive pathways for TMe-lysine⁺

In comparison to acetyl-lysine- H^+ , the decomposition chemistry in TMe-lysine⁺ is more straightforward and is initiated by reactivity at the side-chain. The primary CID loss pathway involves population moving from m/z 189 ($[H_{21}C_9N_2O_2]^+$) \rightarrow m/z 130 ($[H_{12}C_6NO_2]^+$) \rightarrow m/z 84 ($[H_{10}C_5N]^+$), which corresponds to the initial loss of the side-chain followed by loss of $C(OH)_2$ or $H_2O + CO$ from the C-terminus. In

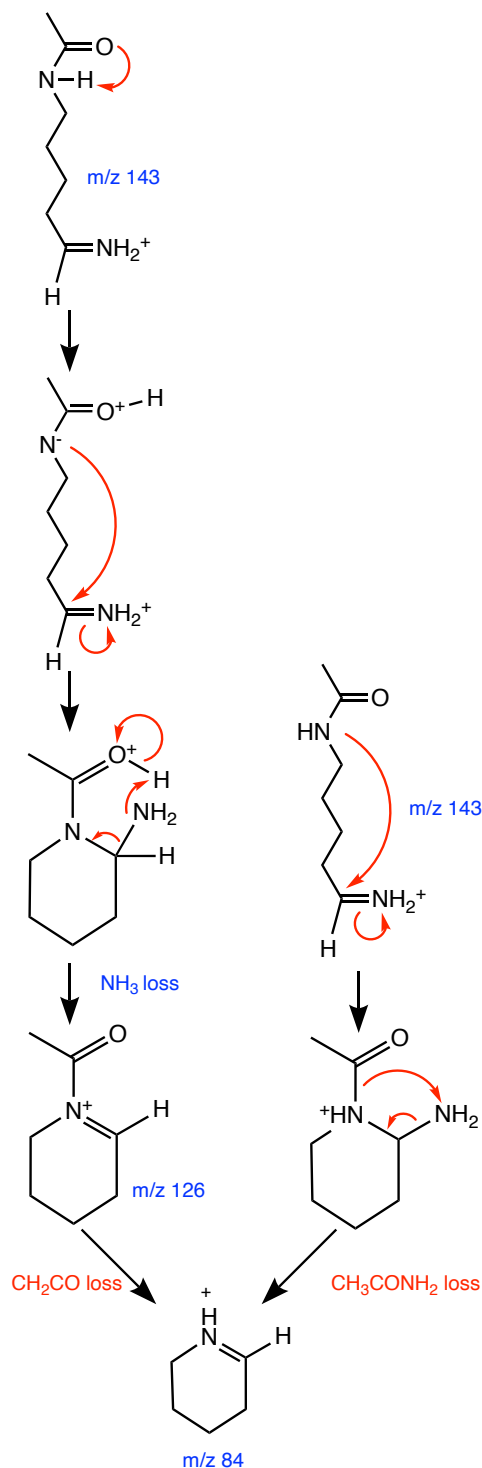


Figure 3: The subsequent and direct mechanisms observed in simulations for the formation of the primary structure of m/z 84 from m/z 143.

contrast to acetyl-lysine- H^+ , the trimethylation of lysine has not deactivated the side-chain as the initial reactive site. Formation of trimethylamine is observed as a byproduct for both the m/z 130 and 84 peaks. Previous experimental work by Hirota *et al*³⁷ also noted the formation of trimethylamine during the CID of TMe-lysine⁺. In addition, our simulations find that the formation of protonated trimethyl amine is now possible and corresponds to m/z 60, although at the internal energies considered here it has a lower population than m/z 130 and 84. The formation of m/z 130 primarily results in the same structure observed in Pathway 1 of our previous work on lysine,¹⁴ and the most common m/z 84 structures also agree with that work. Although our simulations of TMe-lysine⁺ find that the most common structure of m/z 84 is a straight-chain structure, while those for acetyl-lysine- H^+ find a cyclic structure, it is possible this is an artifact of the simulation time scale. Our previous DFT calculations¹⁴ showed that the cyclic structure is more stable by ~ 94 kJ/mol. A minor pathway that is seen in the experiment that is absent in simulations is m/z 144, which corresponds to a mass loss of 42. Given that the loss of ketene was observed in the simulations of acetyl-lysine- H^+ , it is possible that m/z is related to a direct ketene loss in this system.

Summary

Our experimental and computational results have highlighted the decomposition dynamics that are taking place in acetyl-lysine- H^+ vs TMe-lysine⁺. Overall, acetyl-lysine- H^+ is more reactive than TMe-lysine⁺, likely due to the lack of an excess proton in the latter. While both species share several decomposition peaks in their CID spectra, they exhibit different mechanisms to reach these products. In particular, reactivity at the side chain is reduced for acetyl-lysine- H^+ while for TMe-lysine⁺ decomposition is often initiated at the PTM. The reactivity of TMe-lysine⁺ is, in general, more straight forward while acetyl-lysine- H^+ exhibits a more diverse set of reactive pathways. Simulations starting from acetyl-lysine- H^+ largely yielded m/z 143. Through the use of additional simulations that started with this de-

composition product, we obtained a sufficient population of subsequent decomposition products, including the experimentally observed m/z 126 and 84. Again, this highlights that while the initial reactivity in acetyl-lysine- H^+ is higher than TMe-lysine⁺, the subsequent decomposition dynamics, namely those involving the PTM, are hindered via acetylation.

At high energy - either collision energy in experiments, or internal energy in simulations - the primary decomposition product for both species is m/z 84. For TMe-lysine⁺, this product is formed via a mechanism that is similar to that seen in unmodified lysine, namely m/z 186 \rightarrow m/z 130 \rightarrow m/z 84. The acetylation PTM modifies this previously described mechanism by introducing m/z 143 as the new primary first step, with m/z 84 formed through two mechanisms: m/z 186 \rightarrow m/z 143 \rightarrow m/z 126 \rightarrow m/z 84 and m/z 186 \rightarrow m/z 143 \rightarrow m/z 84. It is interesting that while acetyl-lysine- H^+ retains an excess proton, it is TMe-lysine⁺ that retains a similar mechanism to unmodified lysine.

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