Received: 16 November 2017 | Revised: 21 February 2018 | Accepted: 5 March 2018

DOI: 10.1002/rcm.8108

RESEARCH ARTICLE

WILEY Rapid Communications in Mass Spectrometry

Electron transfer dissociation of synthetic and natural peptides containing lanthionine/methyllanthionine bridges

Ashwini B. Dolle[1] | Narasimhappagari Jagadeesh[2] | Suman Bhaumik[3] | Sunita Prakash[4] | Himansu S. Biswal[3] | Konkallu Hanumae Gowd[1]

[1] Department of Chemistry, School of Chemical Sciences, Central University of Karnataka, Kalaburagi 585367, Karnataka, India

[2] Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, Karnataka, India

[3] School of Chemical Sciences, National Institute of Science Education and Research, Bhubaneswar 752050, Odisha, India

[4] Proteomic Facility, Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, Karnataka, India

Correspondence
K. H. Gowd, Department of Chemistry, School of Chemical Sciences, Central University of Karnataka, Kalaburagi 585367, Karnataka, India.
Email: khgowd@cuk.ac.in

Correspondence for computational work
H. S. Biswal, School of Chemical Sciences, National Institute of Science Education and Research, Bhubaneswar 752050, Odisha, India.
Email: himansu@niser.ac.in

Funding information
DST-INSPIRE faculty grant, Grant/Award Number: IFA-11LSBM-03

Rationale: The modes of cleavage of lanthionine/methyllanthionine bridges under electron transfer dissociation (ETD) were investigated using synthetic and natural lantipeptides. Knowledge of the mass spectrometric fragmentation of lanthionine/methyllanthionine bridges may assist in the development of analytical methods for the rapid discovery of new lantibiotics. The present study strengthens the advantage of ETD in the characterization of posttranslational modifications of peptides and proteins.

Methods: Synthetic and natural lantipeptides were obtained by desulfurization of peptide disulfides and cyanogen bromide digestion of the lantibiotic nisin, respectively. These peptides were subjected to electrospray ionization collision-induced dissociation tandem mass spectrometry (CID-MS/MS) and ETD-MS/MS using an HCT ultra ETDII ion trap mass spectrometer. MS^3 CID was performed on the desired product ions to prove cleavage of the lanthionine/methyllanthionine bridge during ETD-MS/MS.

Results: ETD has advantages over CID in the cleavage of the side chain of lanthionine/methyllanthionine bridges. The cleavage of the N–Cα backbone peptide bond followed by C-terminal side chain of the lanthionine bridge results in formation of $c^{\bullet+}$ and z^+ ions. Cleavage at the preceding peptide bond to the C-terminal side chain of lanthionine/methyllanthionine bridges yields specific fragments with the cysteine/methylcysteine thiyl radical and dehydroalanine.

Conclusions: ETD successfully cleaves the lanthionine/methyllanthionine bridges of synthetic and natural lantipeptides. Diagnostic fragment ions of ETD cleavage of lanthionine/methyllanthionine bridges are the N-terminal cysteine/methylcysteine thiyl radical and C-terminal dehydroalanine. Detection of the cysteine/methylcysteine thiyl radical and dehydroalanine in combined ETD-CID-MS may be used for the rapid identification of lantipeptide natural products.

1 | INTRODUCTION

Electron transfer dissociation mass spectrometry (ETD-MS) is emerging as a method of choice for the characterization of posttranslational modifications (PTMs) of cysteine residues in peptides and proteins.[1-8] Common PTMs of cysteine residues in natural peptides are the formation of cysteine disulfides and cysteine monosulfides (or) lanthionine/methyllanthionine bridges. Characterization of cysteine disulfides using ETD-MS/MS is well reported using a series of examples of disulfide-containing polypeptides.[9-12] ETD-MS/MS of peptide disulfides selectively yields c + 33/z − 33 and c + 32/z − 32 ions and also neutral loss associated with perthiyl and sulfhydryl radicals.[10,13] Further, radical-initiated disulfide isomerization was also observed during ETD-MS/MS of disulfide-rich peptides.[13] Although ETD-MS/MS is widely used for characterization of peptide disulfides, less attention has been paid to the lanthionine/methyllanthionine (Lan/MeLan) bridges of lantipeptides.[14] Compared

with disulfides, monosulfide bridges are extremely difficult to cleave using conventional reagents/methods that are generally employed for characterization of disulfides in peptides/proteins. Identification of Lan/MeLan modification indeed is the bottle neck for discovery of new lantibiotics and lantipeptide natural products.[15-17] Heck's group demonstrated the feasibility of cleavage of the Lan/MeLan bridge during electron capture dissociation (ECD)-MS fragmentation of lantibiotic nisin, mersacidin, and lacticin.[14] In the present studies, we have demonstrated the feasibility of cleavage of the Lan/MeLan bridge during ETD-MS fragmentation of synthetic and natural lantipeptides. Combined ETD-CID-MS methods were used to prove the cleavage of the Lan/MeLan bridge. Even though conceptually ECD and ETD share similar features of cleavage of peptide bonds and provide common c/z ions,[3,10,18-21] the less expensive ETD-MS technique may find wide accessibility for the characterization of lantipeptides.[2,4]

Spatola and coworkers demonstrated a facile route for the direct synthesis of cyclic lanthionine peptides by alkali-assisted desulfurization of cyclic peptide disulfides using several examples of synthetic model peptides.[22,23] Base-catalyzed formation of lanthionine in proteins has already been reported by Ringel and coworkers through isolation of mesolanthionine from sodium carbonate treated wool and several alkali-treated proteins.[24,25] Thakur and Balaram reported the mass spectrometric characterization of the diastereomeric lanthionine peptide derived by alkali treatment of a synthetic 14-membered cyclic disulfide peptide.[26] Herein, we have employed the same procedures to obtain the synthetic $_{mut}$Ar1311 lantipeptide and accessed ETD cleavage of the lanthionine bridge. Electron transfer dissociation of the lanthionine bridge of $_{mut}$Ar1311 selectively yields an N-terminal cysteine thiyl radical and C-terminal dehydroalanine which was confirmed through CID of the corresponding c and z ions. To further support cleavage of the lanthionine bridge under ETD conditions and to rule out the influence of the diastereomeric nature of lanthionine on fragmentation, we have achieved ETD cleavage of the Lan/MeLan bridge of a natural lantipeptide derived from the well-known lantibiotic nisin. Chemical digestion of nisin using cyanogen bromide[27] yields two peptide fragments: $_{CNBr}$Nisin1359 and $_{CNBr}$Nisin1932. These fragments have comparable chain lengths with that of synthetic $_{mut}$Ar1311 lantipeptide and may exhibit less overlapping masses for spectral assignments. Interestingly, ETD cleavage of the inter-locking methyllanthionine bridges of $_{CNBr}$Nisin1359 has revealed the occurrence of radical-mediated isomerization of methyllanthionine bridges. Computational studies were further undertaken to provide the support for occurrence of a novel fragmentation pathway. The present studies highlight the advantage of ETD over CID in structural characterization of lantipeptides and may assist in the development of new mass spectrometric methods for detection of lantibiotics.

2 | EXPERIMENTAL

2.1 | Materials

Nisin (N5764) and cyanogen bromide were purchased from Sigma Chemicals. Chromatography grade acetonitrile, formic acid, trifluoroacetic acid (TFA), and liquor ammonia were purchased from S.D. Fine Chemicals. Milli-Q water was used for all the experiments.

2.2 | Synthesis of $_{mut}$Ar1311 lantipeptide

The synthetic peptide $_{mut}$Ar1311 was obtained from the inventory of Prof. P. Balaram (MBU, IISc Bangalore, India). Mass spectrometric characterization of $_{mut}$Ar1311 was recently reported.[28] The observed mass of the peptide (1233 Da; $[M + H]^+$) is consistent with the presence of a cyclic disulfide in $_{mut}$Ar1311. The lyophilized pure peptide (20 nmol) was dissolved in 500 μL of 0.01% TFA containing water. To the same solution 500 μL of liquor ammonia was added, the pH was adjusted to 9, and the mixture was incubated at room temperature for 2–3 days. The reaction was quenched by acidification using TFA and directly infused into the mass spectrometer.

2.3 | Chemical digestion of nisin using cyanogen bromide

Purified nisin (2 mg) was directly dissolved in 10 mL of 70% formic acid. Then a 10-fold excess of cyanogen bromide was added to the sample and incubated at 25°C under an atmosphere of N_2. Samples were drawn at regular time intervals and reactions were quenched by lyophylization. Dried samples were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) on a C_{18} analytical column using a linear gradient of acetonitrile using a Prominence HPLC system (Shimadzu). The flow rate was 1 mL/min and fractions were detected at 226 nm. Purified nisin fragments were further characterized using mass spectrometry.

2.4 | Mass spectrometry

ETD and CID experiments were performed on a HCT ultra PTM Discovery System ETDII ion trap mass spectrometer (Bruker Daltonics, Germany). The samples were analyzed in direct infusion mode and tandem (MS^n) experiments were performed manually. ETD-MS/MS was performed by using 'smart decomposition' which provides low background collision gas to improve formation of product ions. ETD reaction time was 100 ms. The drying gas flow rate for the experiment was 5 L/min, the nebulizer pressure was 10 psi, and the drying gas temperature was 300°C. The fragmentation amplitude was 1 V. MS^3 CID was performed on the desired product ions by collision with helium gas. Data was processed using data analysis software. We have followed the nomenclature of Chu et al. to designate product ions.[29]

2.5 | Calculations

Geometry optimization and frequency calculations of all the reactants and products were carried out using the Turbomole quantum chemistry package[30] at the RI-BP86/def-SVP[31] level. The absence of imaginary frequency in all optimized structures confirmed the minimum energy structures. Single point energy calculations were performed with a higher basis set, i.e. at the RI-BP86/def-TZVP[32] level.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of synthetic $_{mut}$Ar1311 lantipeptide

Figure 1A shows the ESI-MS profiles of $_{mut}$Ar1311 before and after incubation with liq. NH_3. The shift in the mass of the precursor ion upon addition of alkali to the peptide solution is 32 Da, which can be ascribed to the conversion of disulfide into a monosulfide bridge. An additional peak shifted in mass by 49 Da was assigned to concomitant loss of sulfur and ammonia from $_{mut}$Ar1311. The intensity distribution of the parent and lanthionine containing $_{mut}$Ar1311, as shown in Figure 1A, is of the same order $[M + 2H]^{2+} > [M + 3H]^{3+} > [M + H]^{+}$. Figure 1B shows the possible steps involved in the base-catalyzed conversion of disulfide into lanthionine in $_{mut}$Ar1311. Sequential abstraction of C_α protons of Cys3 and Cys8 of $_{mut}$Ar1311 results in the formation of a pair of dehydroalanine and dihydrogen disulfide (H_2S_2).[26] Degradation of dihydrogen disulfide under basic condition leads to the accumulation of hydrogen sulfide (H_2S) in the reaction mixture which facilitate formation of the lanthionine bridge.[26] Addition of H_2S to the two achiral dehydroalanines of $_{mut}$Ar1311 yields a mixture of LL, LD, DL, and DD configured lanthionine bridges. Formation of such stereoisomers of lanthionine in alkali-treated peptide disulfide is well reported with appearance of multiple peaks having the same mass in the HPLC profile.[22,23,26]

3.2 | ETD-MS of synthetic $_{mut}$Ar1311 lantipeptide

Figure 2 shows the CID-MS/MS and ETD-MS/MS spectra of lanthionine-containing $_{mut}$Ar1311. CID of the $[M + 2H]^{2+}$ ion of lanthionine-containing $_{mut}$Ar1311 yields product ions at *m/z* 186, 879 and 1016 (Figure 2A). These masses correspond to y_2, b_8 and b_9 ions resulting from cleavage of the peptide bond outside the lanthionine loop of $_{mut}$Ar1311. In contrast, ETD of the $[M + 3H]^{3+}$ ion of $_{mut}$Ar1311 lantipeptide yields series of 'c' and 'z' ions resulting from cleavage of peptide bonds both inside as well as outside the lanthionine loop. The product ions at *m/z* 245 $[c_2 + 2H]^{+}$, 896 $[c_8 + 2H]^{+}$, 1130 $[c_{10} + 2H]^{+}$, 307 $[z_3 + 2H]^{\bullet+}$, 958 $[z_9 + 2H]^{\bullet+}$, and 1029 $[z_{10} + 2H]^{\bullet+}$ correspond to the backbone cleavage of peptide bonds outside the lanthionine loop of $_{mut}$Ar1311 (Figure 2B) whereas the ions at *m/z* 377 $[z_4 + 2H]^{+}$ and 826 $[c_7 + 2H]^{\bullet+}$ correspond to cleavage of the Met7–Ala^{S}8 peptide bond, which precedes the C-terminal side chain of the lanthionine loop (Figure 2B). In order to observe $[c_7 + 2H]^{\bullet+}$ and $[z_4 + 2H]^{+}$ ions of $_{mut}$Ar1311 lantipeptide, two bonds must be cleaved; the N–Cα bond of the peptide backbone and the side chain of the lanthionine bridge. Kleinnijenhus et al. have observed the formation of $c^{\bullet}$ and z ions due to ECD (electron capture dissociation) cleavage of the peptide bond and the lanthionine bridge of lantibiotics. These authors also documented that exclusive cleavage of the peptide bond close to the C-terminus of the thioether bridge facilitates effective side-chain cleavage of the Lan/MeLan bridge. Xia and coworkers have proposed initial N–Cα peptide bond cleavage followed by breaking of the disulfide bridge during ETD of disulfide-containing polypeptides. Scheme 1 illustrates the possible steps involved in the formation of 'c' and 'z' ions due to cleavage of the peptide bond and the lanthionine bridge of $_{mut}$Ar1311 lantipeptide. ETD of the peptide bond preceding the C-terminal end of the lanthionine bridge results in formation of 'c^{+}' and '$z^{\bullet+}$' ions which are transiently held together by the lanthionine loop. The newly formed '$z^{\bullet+}$' ion contains an unpaired electron on the Cα carbon bearing the C-terminal side chain of the lanthionine bridge, which is available for radical-mediated fragmentation reactions. One of the possible reactions is the radical-initiated cleavage of the lanthionine side chain to yield the N-terminal cysteine thiyl radical and C-terminal dehydroalanine. This step eventually separates and transforms the transiently held 'c^{+}' and '$z^{\bullet+}$' ions into independent '$c^{\bullet+}$' and 'z^{+}' ions, respectively (Scheme 1(a)). If such radical-initiated fragmentation

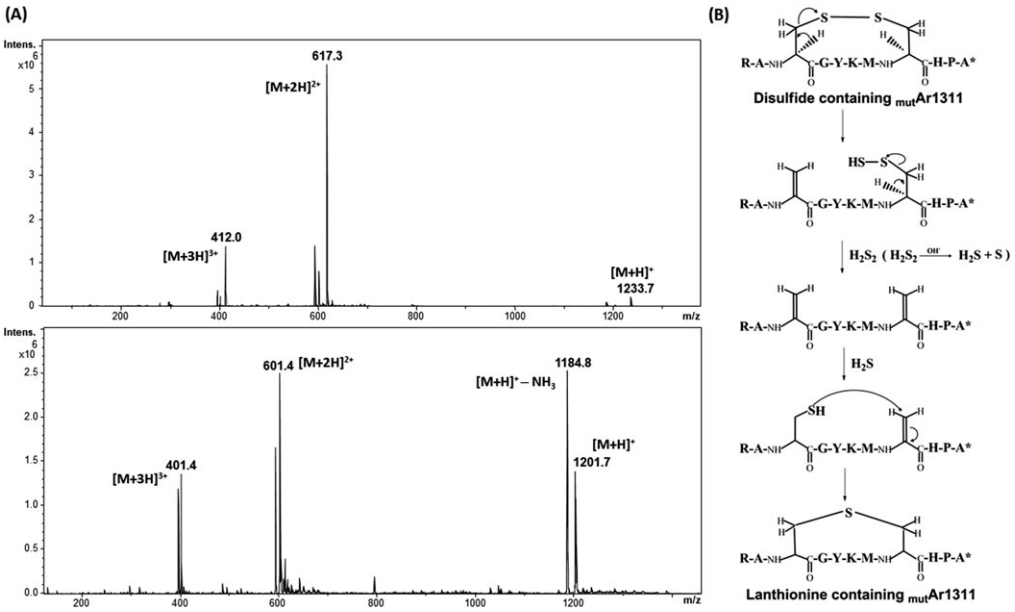


FIGURE 1 Characterization of alkali-induced formation of lanthionine from the cysteine disulfide of $_{mut}$Ar1311. (A) ESI-MS of $_{mut}$Ar1311 (upper panel) and $_{mut}$Ar1311 after alkali treatment (lower panel). (B) Proposed pathway of alkali-induced formation of lanthionine by desulfurization of $_{mut}$Ar1311. Observed charge states and loss of ammonia are indicated

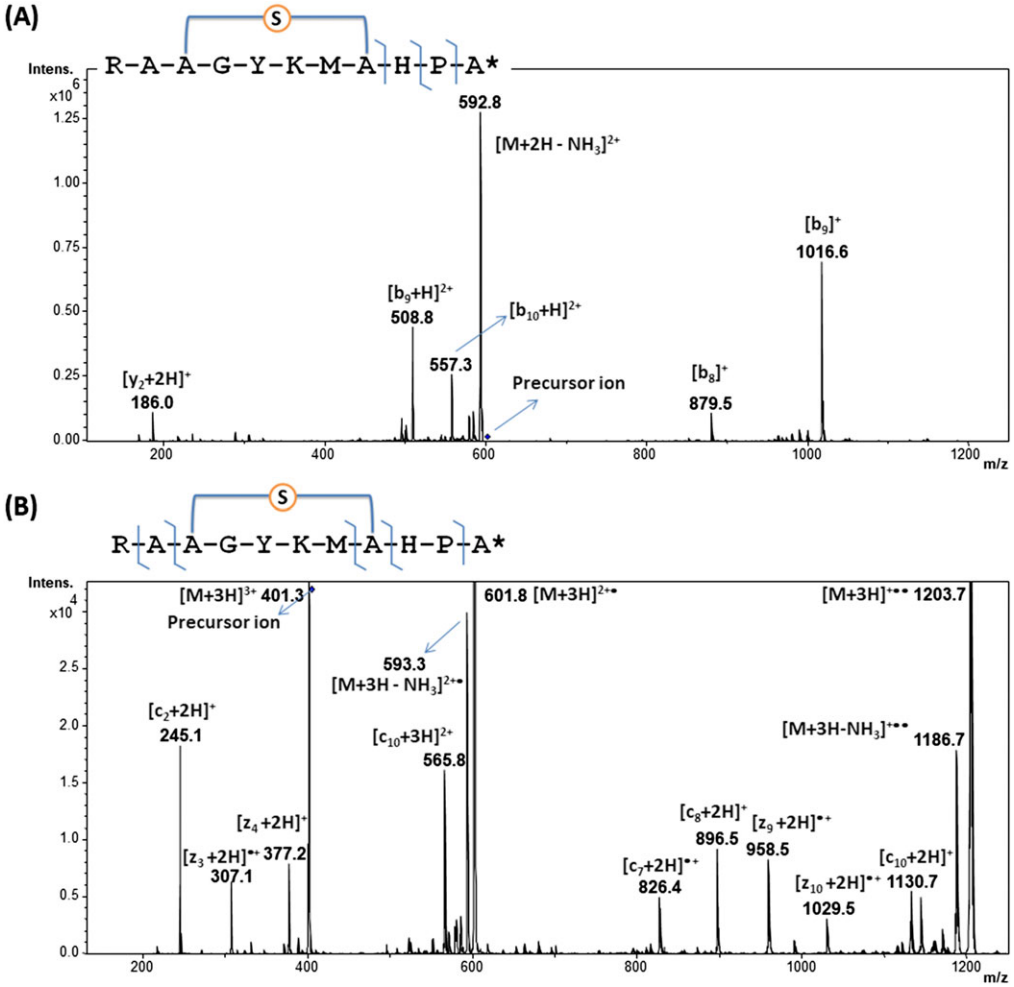
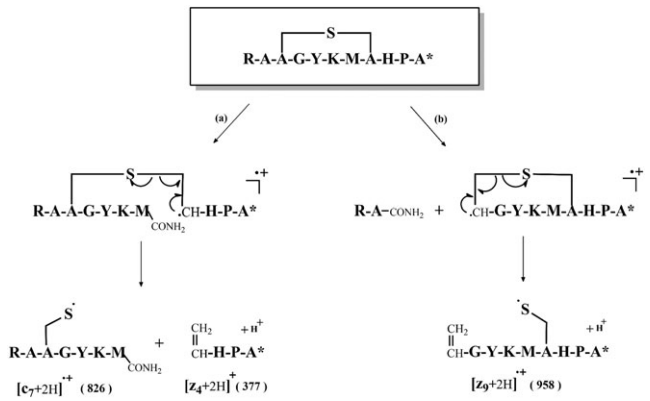


FIGURE 2 Fragmentation of $_{mut}$Ar1311 lantipeptide. (A) CID-MS/MS of the $[M + 2H]^{2+}$ ion of the peptide. (B) ETD-MS/MS of $[M + 3H]^{3+}$ ion of the peptide. Inset shows the observed fragment ions due to cleavage of backbone peptide bonds. The presence of $[c_7 + 2H]^{\bullet+}$ and $[z_4 + 2H]^{+}$ ions indicates cleavage of both the backbone peptide bond and the side chain of the lanthionine bridge. Note the conventional method of representation of the monosulfide (or) lanthionine bridge in the sequence is (Ala-S-Ala) instead of (Cys-S-Cys) [Color figure can be viewed at wileyonlinelibrary.com]



SCHEME 1 Possible modes of cleavage of the Lan bridge during ETD of $_{mut}$Ar1311 lantipeptide. Putative structures of the 'c' and 'z' ions are indicated. Numbers in parentheses indicate the observed mass of the c/z ion in Figure 2B

reactions are involved, one may also anticipate similar radical-induced cleavage of the N-terminal side chain of lanthionine in the case of the '$z^{\bullet+}$' ion formed due to cleavage of the N–Cα peptide bond preceding the N-terminal side chain of the lanthionine bridge (Scheme 1(b)). However, such cleavage is not associated with a change in the mass of the product ion rather it may simply open the structure of the lanthionine loop of $_{mut}$Ar1311 lantipeptide. To provide evidence for the occurrence of radical-initiated cleavage of the lanthionine bridge under ETD, the key product ions $[z_4 + 2H]^{+}$, $[c_7 + 2H]^{\bullet+}$, and $[z_9 + 2H]^{\bullet+}$ of $_{mut}$Ar1311 lantipeptide were subjected to MS^3 CID. Figure 3 shows the CID-MS (MS^3) spectra of $[z_4 + 2H]^{+}$, $[c_7 + 2H]^{\bullet+}$, and $[z_9 + 2H]^{\bullet+}$ ions resulting from ETD cleavage of the lanthionine bridge of $_{mut}$Ar1311 lantipeptide. CID of the $[z_4 + 2H]^{+}$ ion (377.2 Da) yields the $[b_2]^{+}$ ion at *m/z* 192, which is further supported by characteristic loss of carbon monoxide (Figure 3A). An intense peak at *m/z* 186 is consistent with preferential cleavage of the Xxx–Pro bond (where Xxx = His) to yield the $[y_2 + 2H]^{+}$ ion corresponding to the dipeptide PA* (*indicates C-terminal amidation). The observed difference in mass of 55 Da between the b_2 ion and the histidine residue confirms the presence of N-terminal dehydroalanine ($CH(CH_2)CO$) in the $[z_4 + 2H]^{+}$ ion of $_{mut}$Ar1311 lantipeptide. In contrast, CID of the complementary $[c_7 + 2H]^{\bullet+}$ ion (Figure 3B) yields losses of 33 Da (SH) and 46 Da (CH_2S), which are characteristic for the presence of a cysteine thiyl radical in

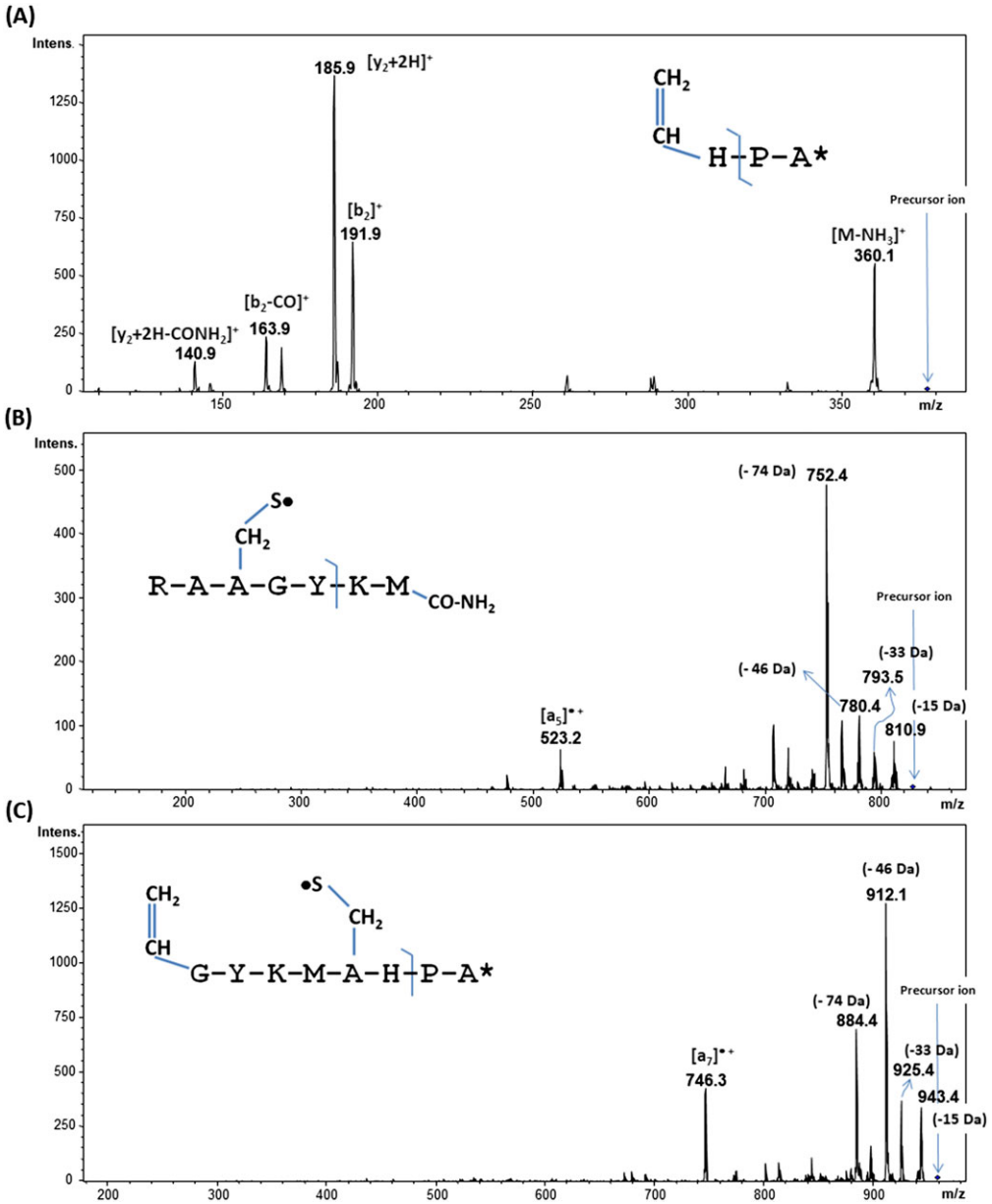


FIGURE 3 CID-MS (MS^3) of product ions resulting from ETD cleavage of the Lan bridge of $_{mut}$Ar1311. (A) $[z_4 + 2H]^+$, (B) $[c_7 + 2H]^{\bullet+}$, and (C) $[z_9 + 2H]^{\bullet+}$ ions derived from ETD of the $[M + 3H]^{3+}$ of lanthionine-containing $_{mut}$Ar1311. Insets in (B) and (C) represent the possible structure of the product ion. Losses of 33 Da and 46 Da were assigned to -SH and $-CH_2S$, respectively. Similarly, loss of 15 Da was assigned to the $-CH_3$ group. The loss of 74 Da was assigned to side-chain loss of methionine minus 1 Da [Color figure can be viewed at wileyonlinelibrary.com]

peptides.[10,13,33] The losses of 15 Da and 74 Da may be assigned to methyl group and side chain loss of methionine minus 1 Da. The presence of the cysteine thiyl radical in the $[c_7 + 2H]^{\bullet+}$ ion confirms the occurrence of radical-mediated cleavage of the lanthionine bridge during ETD of $_{mut}$Ar1311 lantipeptide. The insert in Figure 3B shows a possible structure for the $[c_7 + 2H]^{\bullet+}$ ion. Interestingly, CID of the $[z_9 + 2H]^{\bullet+}$ ion (Figure 3C) also yields losses of 33 Da (SH) and 46 Da (CH_2S), which is consistent with the presence of the cysteine thiyl radical in the $[z_9 + 2H]^{\bullet+}$ ion. The mass equivalence of the open and closed structures of lanthionine in $[z_9 + 2H]^{\bullet+}$ (Scheme 1(b)) may not be of immediate use in the identification of the lanthionine bridge in lantipeptides. However, radical-mediated cleavage of the $[z_9 + 2H]^{\bullet+}$ ion lanthionine bridge results in formation of the N-terminal dehydroalanine and C-terminal cysteine thiyl radical (Scheme 1(b)). The presence of the cysteine thiyl radical in the $[c_7 + 2H]^{\bullet+}$ and $[z_9 + 2H]^{\bullet+}$ ions of $_{mut}$Ar1311 lantipeptide clearly indicates the occurrence of radical-mediated cleavage of the lanthionine bridge during ETD of $_{mut}$Ar1311 lantipeptide.

3.3 | Characterization of cyanogen bromide cleaved fragments of nisin

Nisin is a well-characterized lanthionine and methyllanthionine-containing natural lantipeptide derived from Gram-positive bacteria such as *Lactococcus lactis*.[17,34,35] Nisin contains a lanthionine bridge and four 3-methyllanthionine bridges in the native sequence. The

inter-locked conformation of methyllanthionine bridges is located at the C-terminus of nisin. Natural lantipeptide with a comparable chain length to that of the synthetic $_{mut}$Ar1311 lantipeptide can be obtained through fragmentation of nisin into shorter lantipeptides. The presence of methionine residues, Met-17 and Met-21, permits fragmentation of nisin into shorter lantipeptides through backbone cleavage of the peptide bond using cyanogen bromide.[27,36] Compared to tryptic fragments of nisin, cyanogen bromide cleaved fragments of nisin will have relatively homogeneous distribution of positive charges along the length of short lantipeptides which may be of importance for ETD-MS studies. Purified nisin (Figure S1, supporting information) was treated with cyanogen bromide as described in section 2.3. Figure S2a (supporting information) shows the HPLC elution profile of nisin before and after treatment with cyanogen bromide. The observed masses of fragments eluting at 14.2 min and 20.5 min are 1359 Da and 1932 Da, respectively (Figure S3, supporting information). The shorter nisin lantipeptide with *m/z* 1359 corresponds to the native C-terminus of nisin (Figure S2b, supporting information). The shorter nisin lantipeptide with *m/z*1932 corresponds to the modified N-terminus of nisin with two peptide fragments linked by an inter-molecular methyllanthionine bridge and C-terminus with homoserine lactones (Figure S2b, supporting information). The total number of thioether bridges in the modified N-terminus fragment of nisin is 3: an inter-molecular MeLan bridge and two intramolecular Lan/MeLan bridges. Assignment of structures to the cyanogen bromide cleaved peptide fragments of nisin is in accordance with a previous report by Slootweg et al.[27] From here onwards the cleaved nisin fragments are designated as $_{CNBr}$Nisin1359 and $_{CNBr}$Nisin1932.

3.4 | ETD-MS of natural $_{CNBr}$Nisin1359 lantipeptide

CID-MS/MS of $_{CNBr}$Nisin1359 yields $[b_9]^+$, $[b_{10}]^+$, $[b_{11}]^+$, and $[b_{12}]^+$ ions which arise due to cleavage of the peptide bond outside the MeLan bridges (Figure 4A). The presence of doubly charged $[b_{10}]^+$, $[b_{11}]^+$, and $[b_{12}]^+$ ions is in accordance with N-terminal basic residues in $_{CNBr}$Nisin1359. ETD of $_{CNBr}$Nisin1359 yields several 'c' and 'z' ions due to cleavage of the peptide bond across the chain length of

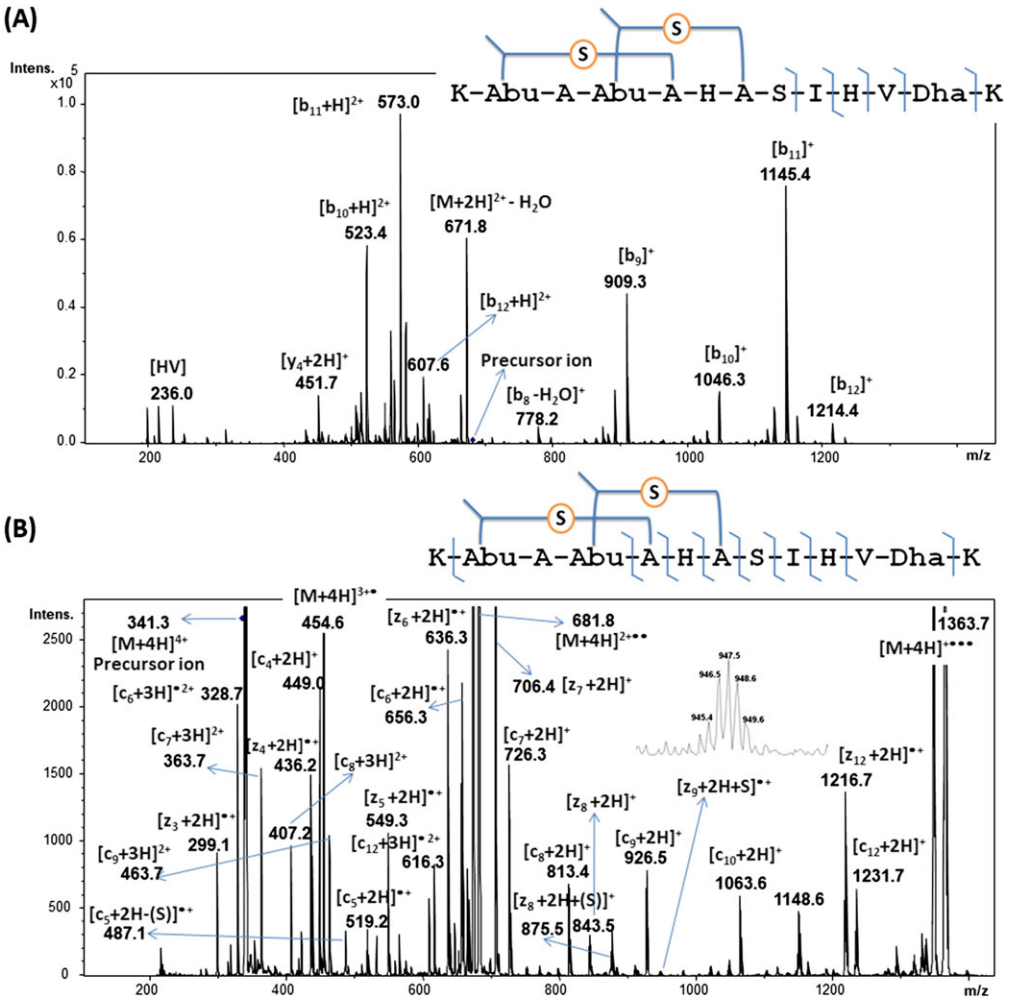
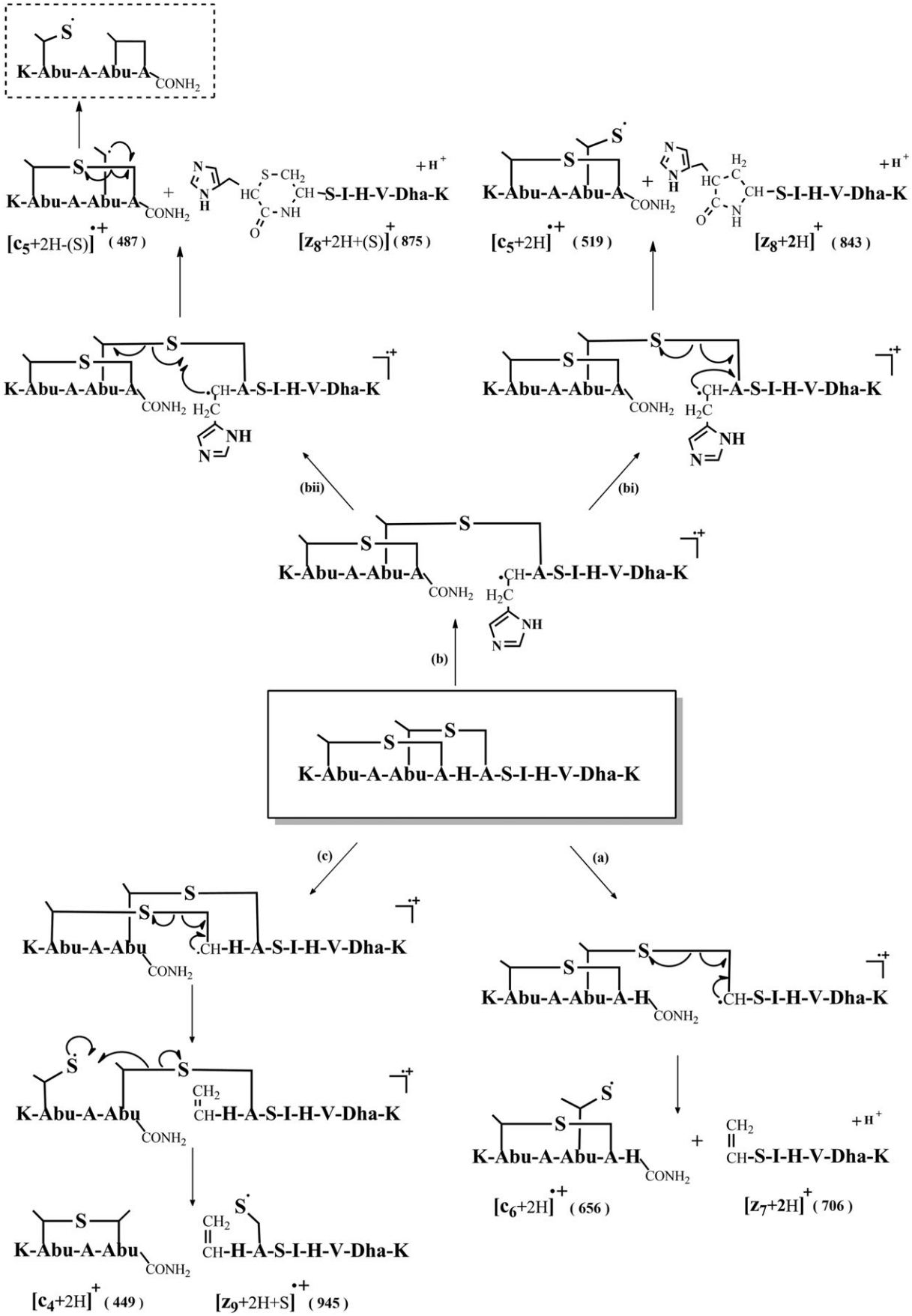


FIGURE 4 Fragmentation of $_{CNBr}$Nisin1359. (A) CID-MS/MS of the $[M + 2H]^{2+}$ ion of the peptide. (B) ETD-MS/MS of the $[M + 4H]^{4+}$ ion of the peptide. Inset shows observed fragment ions due to cleavage of backbone peptide bonds. Presence of $[c_5 + 2H]^{\bullet+}$, $[c_6 + 2H]^{\bullet+}$, $[c_4 + 2H]^+$, $[z_7 + 2H]^+$ and $[z_8 + 2H]^+$ ions indicates cleavage of both the backbone peptide bond and the side chain of the MeLan bridge. Presence of $[c_4 + 2H]^+$ and $[z_9 + 2H + S]^{\bullet+}$ ion indicates radical cascade isomerization of the MeLan bridge. Note that the mass of the deamidated $[M + 4H]^{3+\bullet}$ ion coincides with the mass of the $[c_4 + 2H]^+$ ion [Color figure can be viewed at wileyonlinelibrary.com]

$_{CNBr}$Nisin1359 (Figure 4B). The ions at *m/z* 726, 813, 926, 1063, and 1231 correspond to $[c_7 + 2H]^+$, $[c_8 + 2H]^+$, $[c_9 + 2H]^+$, $[c_{10} + 2H]^+$, and $[c_{12} + 2H]^+$, respectively. Similarly, ions at *m/z* 299, 436, 549, 636, and 1216 correspond to $[z_3 + 2H]^{\bullet+}$, $[z_4 + 2H]^{\bullet+}$, $[z_5 + 2H]^{\bullet+}$, $[z_6 + 2H]^{\bullet+}$, and $[z_{12} + 2H]^{\bullet+}$, respectively. These 'c' and 'z' ions arise due to cleavage of the peptide bond outside the MeLan bridges of $_{CNBr}$Nisin1359 (Figure 4B). Ions at *m/z* 449, 519, 656, 706, 843, and 945, corresponding to $[c_4 + 2H]^+$, $[c_5 + 2H]^{\bullet+}$, $[c_6 + 2H]^{\bullet+}$, $[z_7 + 2H]^+$, $[z_8 + 2H]^+$, and $[z_9 + 2H + S]^{\bullet+}$, arise due to cleavage of both the peptide bond and the methyllanthionine bridges of $_{CNBr}$Nisin1359 (Figure 4B). Note that the mass of the $[c_4 + 2H]^+$ ion at *m/z* 449 also coincides with the mass of the deamidated $[M + 4H]^{3+\bullet}$ ion. Scheme 2 provides the rationale for formation of 'c' and 'z' ions due to cleavage of the peptide bond and the MeLan bridges of $_{CNBr}$Nisin1359. ETD of the His6–Ala^{S}7 peptide bond leads to the formation of a C_α radical bearing the C-terminal side chain of the second thioether bridge. Radical migration cleaves the thioether bridge leading to formation of the c_6 ion with a cysteine thiyl radical and the $[z_7 + 2H]^+$ ion with N-terminal dehydroalanine (Scheme 2(a)). Cleavage of the Ala^{S}5–His6 peptide bond, which is penultimate to the C-terminal side chain of the second MeLan bridge of $_{CNBr}$Nisin1359, leads to the formation of the C_α radical of the histidine residue. In this case, the radical-mediated cleavage of the side chain of the thioether bridge likely involves cyclization. Xia and Tureček have reported the formation of a cyclic 'z' ion during ETD of an intra-chain disulfide peptide.[7,10,13] In the same way, the histidine Cα radical may cleave the side chain of the second thioether bridge in two different modes due to the extended structure by a peptide bond from the C-terminal side chain of the thioether bridge. As shown in Scheme 2(bi), the radical-mediated cleavage leads to the formation of the $[c_5 + 2H]^{\bullet+}$ ion with the methylcysteine thyil radical and the $[z_8 + 2H]^+$ ion with the N-terminal five-membered cyclic amide. As shown in Scheme 2(bii), the radical-mediated cleavage leads to the formation of the $[c_5 + 2H–(S)]^{\bullet+}$ ion with the radical and the $[z_8 + 2H+(S)]^+$ ion with the N-terminal six-membered cyclic amide. The stability of the six-membered heterocyclic non-aromatic ring due to the absence of angle strain in the chair form and the common occurrence of cyclic five-membered b ions under the MS conditions prompted us to propose the putative structures of cyclic amide of $[z_8 + 2H]^+$ and $[z_8 + 2H+(S)]^+$ ions. Kleinnijenhus et al... also reported that ECD cleavage of the penultimate peptide bond to the C-terminal thioether of nisin A and nisin Z yields 'c$^\bullet$' and 'z' ions. The $[c_5 + 2H]^{\bullet+}$ and $[c_6 + 2H]^{\bullet+}$ ions of $_{CNBr}$Nisin1359 contain a MeLan bridge and a methylcysteine thiyl radical. Tan et al have reported thiyl/perthiyl radical-mediated isomerization of disulfide bonds and the presence of multiple structural isomers for radical-containing z_n ions.[13] They have also reported that the thiyl/perthiyl radical may attack the C–S bond giving rise to new C–S bonds and another thiyl/perthiyl radical which is favourable due to the thermodynamically neutral reaction. Similarly, we anticipate that the initially formed thiyl radical of the $[c_5 + 2H]^{\bullet+}$ and $[c_6 + 2H]^{\bullet+}$ ions may participate in the radical cascade reaction with the first methyllanthionine bridge and may generate a new thioether bridge and thiyl radical. Thus isomeric structures may be possible for the $[c_5 + 2H]^{\bullet+}$ and $[c_6 + 2H]^{\bullet+}$ ions.[7,8,37-40] The formation of $[c_4 + 2H]^+$ and $[z_9 + 2H + S]^{\bullet+}$ ions indicate cleavage of the Abu^{S}4–Ala^{S}5 peptide bond and cleavage and isomerization of the MeLan bridge of $_{CNBr}$Nisin1359. In order to obtain these ions, the peptide bond and two MeLan bridges must be cleaved which is rationalized in Scheme 2(c). Cleavage of the N-Cα bond of Abu^{S}4-Ala^{S}5 leads to the formation of Cα radical bearing the C-terminal side chain of the first MeLan bridge. Newly formed '$c^{+\prime}$' and '$z^{\bullet+\prime}$' ions are held together by both the MeLan bridges. A radical-initiated reaction cleaves the C-terminal side chain of the first MeLan bridge leading to formation of the methylcysteine thiyl radical and dehydroalanine. The formed '$c^{\bullet+\prime}$' and '$z^{+\prime}$' ions are transiently held together by the second MeLan bridge. Further, we hypothesize that the methylcysteine thiyl radical mediated cascade reaction, similar to the observation of Tan et al[13] of a radical cascade reaction in disulfide regiomers, may lead to isomerization of the thioether bridge eventually resulting in formation of '$c^{+\prime}$' and '$z^{\bullet+\prime}$' ions. Thus, $[c_4 + 2H]^+$ and $[z_9 + 2H + S]^{\bullet+}$ ions may contain a cation and a radical cation, respectively. The product ions $[z_7 + 2H]^+$ and the complementary $[c_6 + 2H]^{\bullet+}$ ion yield fragments upon CID (Figure 5), permitting evaluation of the occurrence of radical-mediated cleavage of the MeLan bridge of $_{CNBr}$Nisin1359. Figure 5A shows the CID-MS (MS^3) spectrum of the $[z_7 + 2H]^+$ ion. Occurrence of product ions at *m/z* 392, 491, and 560 corresponding to $[b_4]^+$, $[b_5]^+$, $[b_6]^+$ ions are consistent with the presence of the N-terminal dehydroalanine in the $[z_7 + 2H]^+$ ion. MS^3 of the complementary $[c_6 + 2H]^{\bullet+}$ ion (Figure 5B) results in the losses of 33 Da (SH), 45 Da (CHS,) and 60 Da (CH_3CHS), which are consistent with the presence of the β-methyl cysteine thiyl radical in the $[c_6 + 2H]^{\bullet+}$ ion. Interestingly, CID-MS (MS^3) of the doubly charged $[c_6 + 2H]^{\bullet+}$ ion (Figure 5C) also yields a neutral loss corresponding to the -SH, -CHS, and -CH_3CHS groups of the β-methyl cysteine thiyl radical. These results confirm the occurrence of radical-mediated cleavage of the MeLan bridge during ETD of $_{CNBr}$Nisin1359. Predominant neutral losses associated with the methyl cysteine thiyl radical (Figures 5B and 5C) posing difficulties in proving the occurrence of the radical-mediated formation of isomeric structures of the $[c_6 + 2H]^{\bullet+}$ ion. The product ions $[c_5 + 2H]^{\bullet+}$, $[c_5 + 2H–(S)]^{\bullet+}$, and $[z_8 + 2H+(S)]^+$ yield less intense fragment ions (Figure S4, supporting information). Comparison of the CID-MS (MS^3) of 487 and 519 confirms the presence of sulfur in the $[c_5 + 2H]^{\bullet+}$ ion of $_{CNBr}$Nisin1359. These observations support the possibility of involvement of cyclization during the side-chain cleavage of the thioether bridge by the Cα radical of the histidine residue formed by ETD of the Ala^{S}5–His6 peptide bond. However, poor fragmentation of the $[z_8 + 2H+(S)]^+$ ion (Figure S4c, supporting information) makes it difficult to confirm the presence of the cyclic six-membered ring at the N-terminus of the $[z_8 + 2H+(S)]^+$ ion of $_{CNBr}$Nisin1359.

3.5 | ETD-MS of modified natural $_{CNBr}$Nisin1932 lantipeptide

Figure S5a (supporting information) shows the CID-MS/MS spectrum of modified natural $_{CNBr}$Nisin1932. The presence of the fragment ions $[y_{10} + 2H]^+$, $[b_{13}]^+$, $[y_{15} + 3H]^{2+}$, and $[b_{16} + H]^{2+}$ are consistent with the sequence of modified $_{CNBr}$Nisin1932. The presence of the $[y_9 + 2H]^+$+S ion in Figure S5a (supporting information) indicates cleavage of the Abu^{S}8–Pro9 peptide bond and the second methyllanthionine bridge of modified $_{CNBr}$Nisin1932.



SCHEME 2 Possible modes of cleavage of MeLan bridges during ETD of $_{CNBr}$Nisin1359. Putative structures of 'c' and 'z' ions are indicated. The $[z_9 + 2H + S]^{\bullet+}$ ion indicates the isomerization of methyllanthionine bridges during ETD-MS/MS of $_{CNBr}$Nisin1359. Numbers in parentheses indicate the observed mass of the c/z ion in Figure 4B. The assigned structure shown in the dotted rectangle for the $[c_5 + 2H\text{-}(S)]^{\bullet+}$ ion is based on computational studies

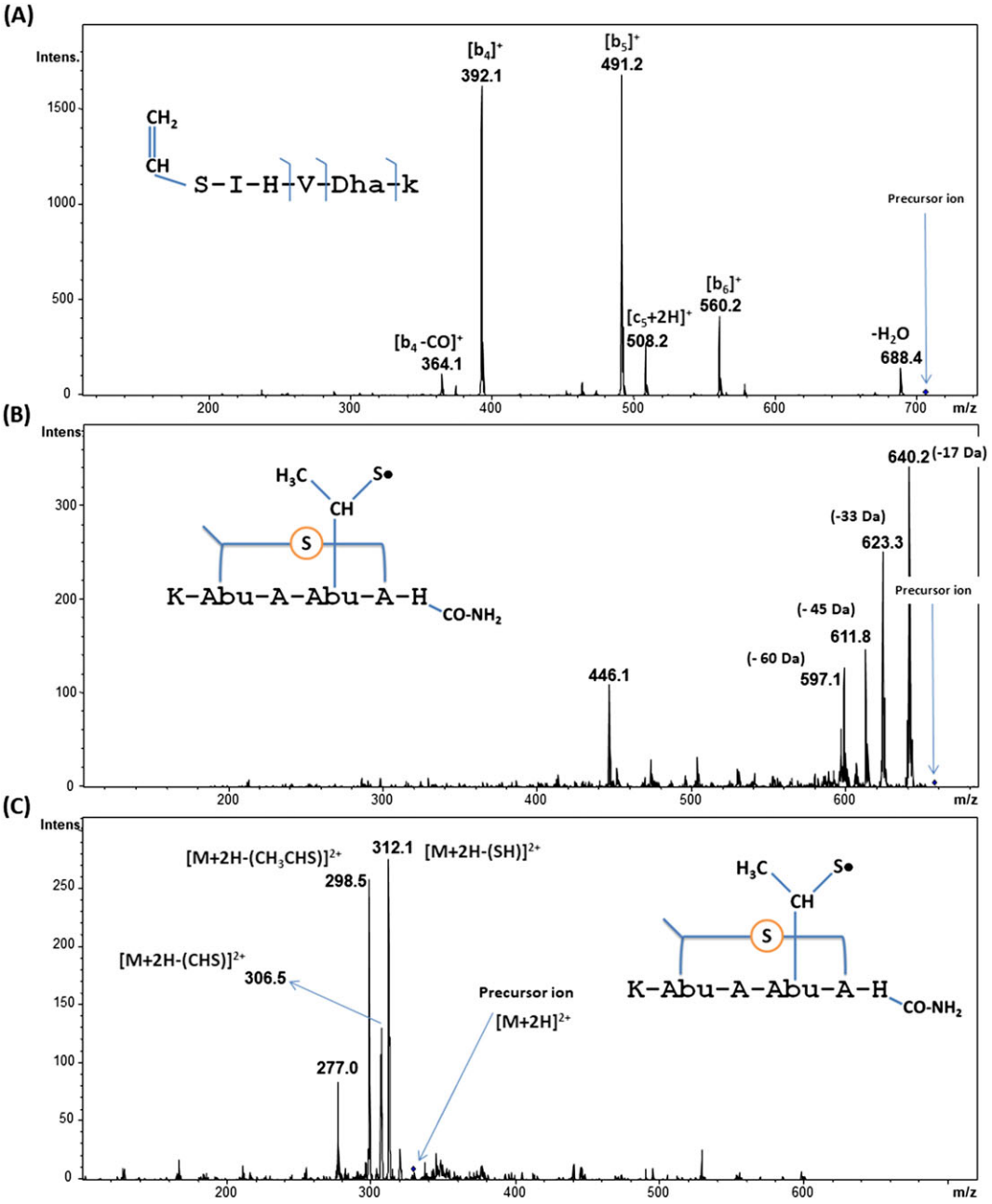


FIGURE 5 CID-MS (MS^3) of product ions resulting from ETD cleavage of the MeLan bridge of $_{CNBr}$Nisin1359. (A) $[z_7 + 2H]^+$, (B) $[c_6 + 2H]^{\bullet+}$, and (C) $[c_6 + 3H]^{\bullet 2+}$ ions derived from ETD of the $[M + 4H]^{4+}$ ion of $_{CNBr}$Nisin1359. Insets in (B) and (C) represent the possible structure of the product ion. Losses of 33 Da, 45 Da, and 60 Da can be assigned to -SH, -CHS, and -$SCHCH_3$, respectively [Color figure can be viewed at wileyonlinelibrary.com]

However, CID of the first Lan bridge and the inter-molecular MeLan bridge is not apparent in the fragmentation spectrum (Figure S4a, supporting information). Figure S4b shows the ETD-MS/MS spectrum of modified natural $_{CNBr}$Nisin1932. It is evident from Figure S4b that the $[z_5 + 2H]^{\bullet+}$, $[z_6 + 2H]^{\bullet+}$, $[z_{10} + 2H]^{\bullet+}$, $[z_{15} + 2H]^{\bullet+}$, $[c_{11} + 2H]^+$, $[c_{12} + 2H]^+$, $[c_{15} + 2H]^+$, and $[c_{16} + 2H]^+$ xions result from cleavage of the peptide bonds outside the Lan/MeLan bridges and the $[z_7 + 2H]^+$ and $[z_{11} + 2H]^+$ ions correspond to cleavage of the peptide bonds inside the Lan/MeLan bridge of modified $_{CNBr}$Nisin1932 (Figure S5b, supporting information). Figure S5 (supporting information) shows the putative structures of the $[z_7 + 2H]^+$ and $[z_{11} + 2H]^+$ ions resulting from cleavage of both the peptide bond and the Lan/MeLan bridge. The ion at *m/z* 983 corresponds to the $[z_7 + 2H]^+$ ion resulting from cleavage of the Gly10–Ala^S11 peptide bond and the C-terminal side chain of the MeLan bridge. Similarly, the ion at *m/z* 1323 corresponds to the $[z_{11} + 2H]^+$ ion resulting from cleavage of the Leu6–Ala^S7 peptide bond and the C-terminal side chain of the Lan bridge. As shown in Figure S5 (supporting information), the $[z_7 + 2H]^+$ and $[z_{11} + 2H]^+$ ions contain N-terminal dehydroalanine. The mass at *m/z* 1590 was assigned to the cleavage of the intermolecular MeLan bridge of modified $_{CNBr}$Nisin1932 (Figure S6, supporting information). Cleavage of the Gly1-Asn2 peptide bond of the shorter peptide unit of modified $_{CNBr}$Nisin1932, followed by radical-initiated cleavage of the inter-thioether bridge, may yield a heavier peptide chain with the cysteine thiyl radical (Figure S6).

3.6 | Computational studies on radical-mediated side-chain cleavage of the MeLan bridges

To provide support for the formation of cyclic structures in product ions during radical-mediated side-chain cleavage of MeLan bridge and rearrangement of MeLan bridges by the newly formed thiyl radical, structural calculations and estimation of energy differences between desired ions was undertaken by a computational method. Lantipeptide (Abu^{S1}-Ala-Abu^{S2}-Ala^{S1}-His-Ala^{S2} + 2H)$^{2+}$, derived by truncation of extended amino acid residues outside the MeLan bridges of $_{CNBr}$Nisin1359, was chosen as the model for computational studies. It has been proved by Tureček and coworkers through computational studies that the cleavage of the N–Cα peptide bond under ETD is energetically favourable and largely independent of the nature of the side chain of the peptide.[7,20] It is apparent from Figures 2 and 4 that more peptide bonds are cleaved under ETD-MS/MS compared to CID-MS/MS suggesting cleavage of the N-Cα peptide bond of the lantipeptide is also an energetically favourable event. Thus, differences in energy between the intermediate and product ions were estimated to access the feasibility of cyclization during side-chain cleavage of the MeLan bridge and thiyl radical mediated rearrangement of the thioether bridge. The electronic structures of the intermediate and product ions in the gaseous phase were deduced by standard density functional theory (DFT) procedures as described in section 2.5 and (2S,3S,6R)[17,27] was the configuration of the 3-methyllanthionine bridge. Figure 6 shows the structures of the lowest energy conformers of the intermediate and product ions of the model lantipeptide for the anticipated fragmentation pathway of Scheme 2(b). It is evident from Figure 6 that the lowest energy conformers are stabilized through hydrogen bonding; particularly, the protonated histidine residue is H-bonded to the peptide carbonyl in the five-membered and six-membered cyclic structures of the product ions. This feature is consistent with a previous report by Moss et al. on assigning structures to gas-phase histidine peptide radical cations by computational studies.[41] Interestingly, hydrogen bonds involving sulfur atoms were also evident in the structures of both the intermediate and product ions. A combination of laser spectroscopy and quantum mechanical calculations has suggested the sulfur centric hydrogen bonds are as strong as conventional hydrogen bonds.[42,43] Table 1 provides the energy differences between the intermediate and product ions of the anticipated fragmentation pathways of Scheme 2. It is apparent from Table 1 that radical-mediated side-chain cleavage of the MeLan bridge resulting in a 'z^{+}' ion with five-membered cyclic amide and a '$c^{\bullet}$' ion with thiyl radical is an exothermic and favourable reaction. The radical-mediated side-chain cleavage of the MeLan bridge to yield a cyclic six-membered 'z^{+}' ion and a secondary carbon radical containing '$c^{\bullet}$' ion is an endothermic reaction. However, radical migration from the secondary carbon to the sulfur atom involving formation of a cyclic six-membered ring in the '$c^{\bullet}$' ion eventually leads to an exothermic and favourable reaction. These observations suggest that formation of the thiyl radical is an energetically favourable fragmentation pathway and a radical-mediated cascade reaction may occur during formation of the product ions. Interestingly, the methylcysteine thiyl radical mediated isomerization of the MeLan bridge is energetically not favourable which may be a reason for the observation of a low intensity $[z_9 + 2H + S]^{\bullet+}$ product ion during ETD-MS/MS of $_{CNBr}$Nisin1359. Computational studies with

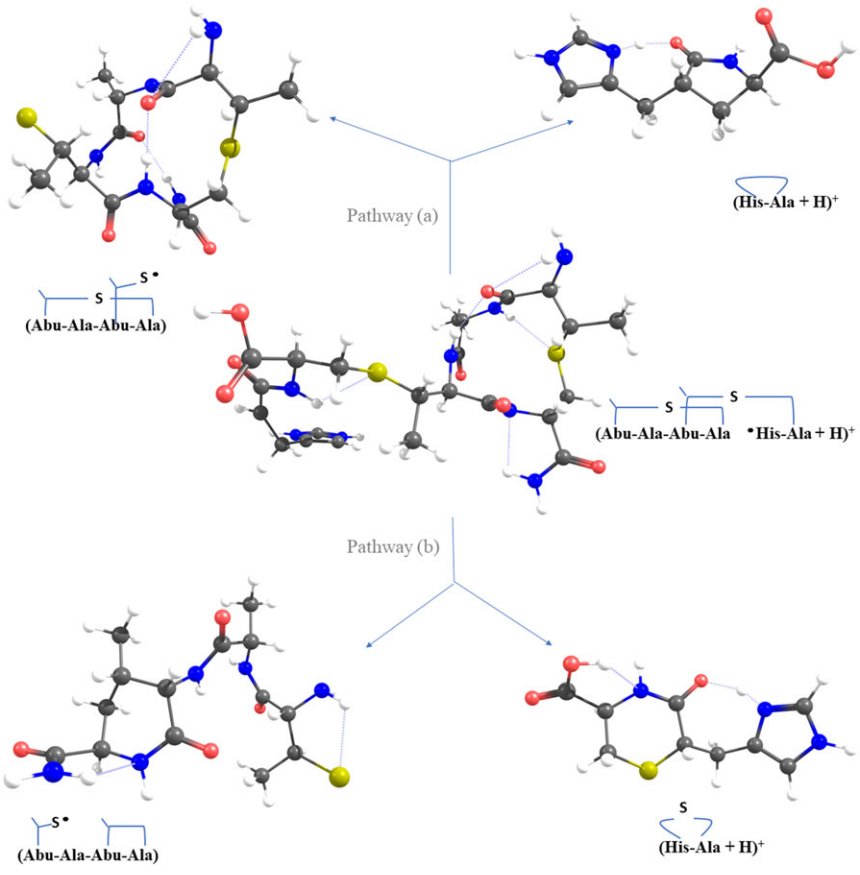


FIGURE 6 Representative structures of intermediate and product ions of the model lantipeptide (Abu^{S1}-Ala-Abu^{S2}-Ala^{S1}-His-Ala^{S2} + H)$^{\bullet+}$ for the anticipated fragmentation pathway proposed in Scheme 2(b). Pathways (a) and (b) indicate the involvement of formation of five- and six-membered cyclic amide containing product ions, respectively. Hydrogen bonds are indicated by a dotted line. The primary structures of the intermediate and product ions are also indicated [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Differences in the binding energies of intermediate and product ions of model lantipeptide for anticipated fragmentation pathways

Intermediate ion	Product ions		BE ($kJ.mol^{-1}$)
	'c' ion	'z' ion	
$(Abu^{S1}$-Ala-Abu^{S2}-$Ala^{S1}{}_b$ ^{a}(CH)His-Ala^{S2} + H)$^+$	Abu^{S1}-Ala-Abu(S•)-$Ala^{S1}{}_b$	(−His (CH_2) Ala− + H)$^+$	−83.03
$(Abu^{S1}$-Ala-Abu^{S2}-$Ala^{S1}{}_b$ ^{a}(CH)His-Ala^{S2} + H)$^+$	Abu(S•)-Ala-Abu ^{S2}-Ala $^{S1}{}_b$	(−His (CH_2) Ala− + H)$^+$	+11.95
$(Abu^{S1}$-Ala-Abu^{S2}-$Ala^{S1}{}_b$ ^{a}(CH)His-Ala^{S2} + H)$^+$	Abu^{S1}-Ala-Abu$((CH_3)$CH•)-$Ala^{S1}{}_b$	(−His (S-CH_2) Ala− + H)$^+$	+22.20
$(Abu^{S1}$-Ala-Abu^{S2}-$Ala^{S1}{}_b$ ^{a}(CH)His-Ala^{S2} + H)$^+$	Abu(S•)-Ala-(−Abu$((CH_3)$CH)-Ala−)$_b$	(−His (S-CH_2) Ala− + H)$^+$	−57.24
$(Abu^{S1}$-Ala-$Abu^{S2}{}_b$ ^{a}(CH)Ala^{S1}-His-Ala^{S2} + H)$^+$	Abu^{S1}-Ala-$Abu^{S2}{}_b$	(Dha-His-Ala(S•) + H)$^+$	+36.72
$(Abu(S)^a$-Ala-$Abu^{S2}{}_b$ Dha-His-Ala^{S2} + H)$^+$	Abu^{S1}-Ala-$Abu^{S2}{}_b$	(Dha-His-Ala(S•) + H)$^+$	+23.52

[a]Indicates location of the radical in the intermediate ion.

[b]Indicates the presence of -$CONH_2$.

S1-S1 and S2-S2 indicate the 3-methyllanthionine bridge.

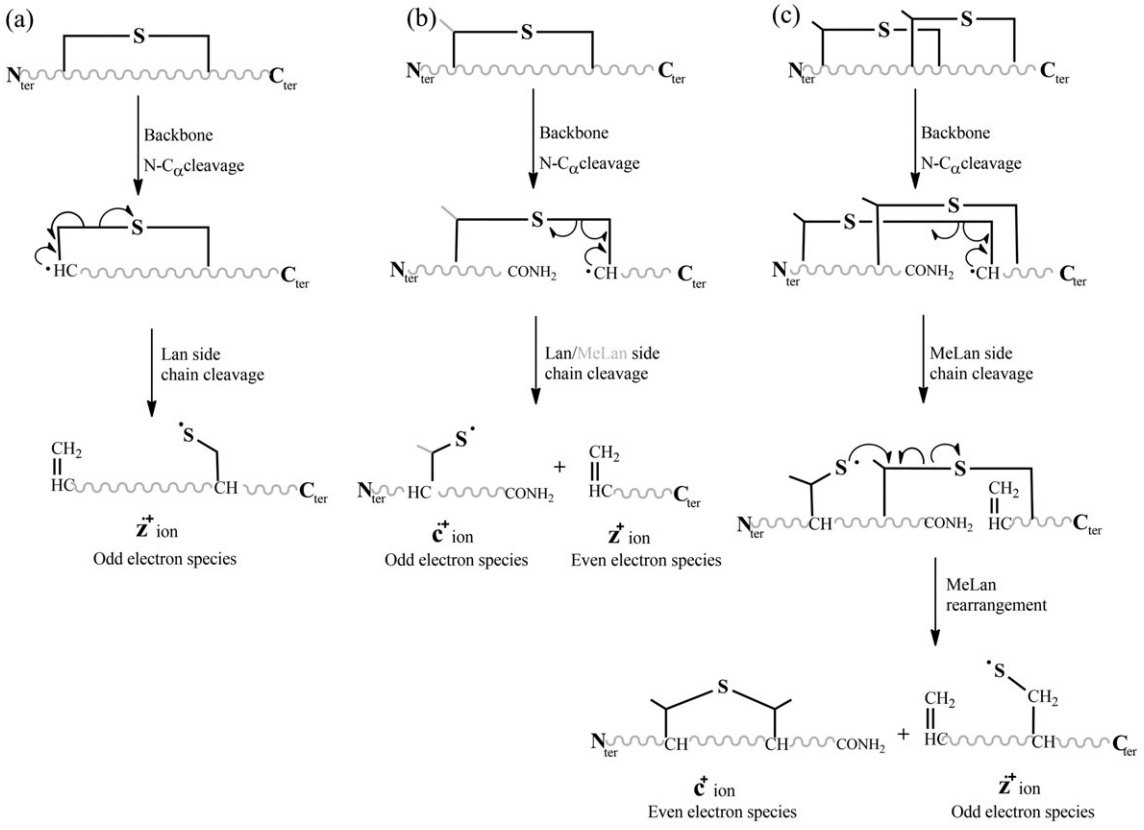
S1-S2 and S2-S1 in product 'c' ions indicate 3,3′-dimethyllanthionine and 3-methyllanthionine bridge, respectively.

The non-native lanthionine bridges in product ions are formed due to isomerisation of the thioether bond.

the model lantipeptide has allowed us to assign a new structure for the $[c_5 + 2H\text{-}(S)]^{\bullet+}$ product ion (insert of Scheme 2) formed during ETD-MS/MS of $_{CNBr}$Nisin1359.

3.7 | Radical-initiated cleavage of intramolecular Lan/MeLan bridges

Characteristic ETD fragment ions of Lan/MeLan bridges can be observed due to cleavage of the N–Cα backbone peptide bond and the side chain of the thioether bridge. Either of these bonds may be cleaved first, resulting in the formation of a radical center which subsequently initiates cascade reactions leading to the formation of c/z ions. If the thioether bond cleaves first, it results in the formation of cysteine/methylcysteine thiol and alanine methyl radical which eventually cleaves the peptide bond to yield $c^{\bullet+}$ and z^+ ions. If such reactions are taking place in the present studies, we should not observe formation of the cysteine/methylcysteine thiyl radical and specificity of cleavage of the peptide bond closest to the C-terminus of the Lan/MeLan bridge. The formation of the cysteine/methylcysteine thiyl radical and dehydroalanine indicates the more likely event would be initial cleavage of the N–Cα backbone peptide bond followed by radical-initiated cleavage of the side chain of the



SCHEME 3 Summary of modes of cleavage of Lan and MeLan bridges during ETD of synthetic and natural lantipeptides. (a) Cleavage of the N-terminal side chain of the Lan bridge, (b) cleavage of the C-terminal side chain of the Lan/MeLan bridge, and (c) radical cascade rearrangement of MeLan bridges. Possible nature of c/z ions is indicated

Lan/MeLan bridge. In the recent report on ETD cleavage of peptide disulfides by Xia and coworkers, cleavage of the N-Cα backbone peptide bond was proposed prior to side-chain cleavage of cysteine disulfides. These observations support the possibility of initial cleavage of the N–Cα backbone peptide bond prior to the thioether bridge during ETD of the Lan/MeLan bridge. Thus the mode of the radical-initiated side-chain cleavage of the Lan/MeLan bridge may be viewed in the context of cleavage of the backbone peptide bond preceding the lanthionine loop and within the lanthionine loop (Scheme 3). Note that the cleavage of the backbone peptide bond succeeding the lanthionine loop results in formation of radical ions on the linear peptide fragment. The cleavage of the peptide bond preceding the N-terminal side chain of the lanthionine loop results in formation of an '$z^{\bullet+}$' ion with an open structure of the lanthionine loop (Scheme 3(a)). The mass of the open structure is indistinguishable from the closed structure; thus it is of less significance and immediate use in the identification of the Lan/MeLan bridge of the lantipeptide. The cleavage of the peptide bond within the lanthionine loop, except in the case of the peptide bond preceding the C-terminal side chain of the lanthionine loop, may require cyclization to break the side chain of the lanthionine loop. Formation of $[c_5 + 2H]^{\bullet+}$, $[c_5 + 2H\text{-}(S)]^{\bullet+}$, $[z_8 + 2H]^{+}$, and $[z_8 + 2H+(S)]^{+}$ ions of $_{CNBr}$Nisin1359 supports a possible involvement of cyclization during the radical-initiated cleavage of the C-terminal side chain of the MeLan bridge (Schemes 2(bi) and Scheme 2(bii)). However, we have rarely observed such cleavage of the C-terminal side chain of Lan/MeLan bridges in both synthetic and natural lantipeptides. Interestingly, if the initial peptide bond proceeding the C-terminal side chain of the lanthionine loop cleaves, the entropically unfavourable step of cyclization is not involved with side-chain cleavage of the Lan/MeLan bridge (Scheme 3(b)). This could be a possible reason for observing specificity in the cleavage of the Lan/MeLan bridge during the ETD of lantipeptides. All the peptide bonds preceding the C-terminal side chain of Lan/MeLan bridges indeed cleaved to yield '$c^{\bullet+}$' and 'z^{+}' ions. In the case of peptide bond cleavage in overlapping regions of lanthionine loops, radical cascade isomerization of MeLan bridges may yield $c^{+}/z^{\bullet+}$ ions (Scheme 3(c)). This feature further supports the reactivity of the cysteine/methylcysteine thiyl radical and suggests the possibility of having more than one structure for the corresponding radical-containing product ions. Formation of the cysteine/methylcysteine thiyl radical and dehydroalanine during ETD of lantipeptides indicates that they may be used as signature for identification of Lan/MeLan bridges in lantibiotics.

4 | CONCLUSIONS

Electron transfer dissociation mass spectrometry successfully cleaves the Lan/MeLan bridges of both synthetic and natural lantipeptides. Initial N–Cα backbone peptide bond cleavage followed by the C-terminal side chain of the Lan/MeLan bridge yields unconventional $c^{\bullet+}$ and z^{+} ions. In the case of inter-locked conformation of MeLan bridges, ETD cleavage of the peptide bond in the overlapped region leads to radical-mediated isomerization of MeLan bridges. Specificity in cleavage at the C-terminal end of the Lan/MeLan bridge results in formation of an N-terminal cysteine thiyl radical and C-terminal dehydroalanine. Identification of the cysteine/methylcysteine thiyl radical through combined ETD-CID-MS indicates the presence of Lan/MeLan bridges in peptides. Facile reduction/alkylation experiments coupled to mass spectrometry[44,45] helps to distinguish disulfides from Lan/MeLan bridges permitting accelerated mining of lantipeptide natural products.

ACKNOWLEDGEMENTS

This article is dedicated to Prof. P. Balaram (MBU, IISc, Bangalore, India) for his contribution to the field of mass spectrometry. Ms. Ashwini is supported by a Junior Research Fellowship (JRF) award from the University Grant Commission (UGC), India. Mr. Jagadeesh is supported by the IAS-INSA-NASI summer fellowship programme. We acknowledge Dr. Basavprabhu for proof-reading the manuscript. We also acknowledge Mr. Santhosh Upadhyaya from Bruker for timely help with mass spectrometry. This research work is supported by the DST-INSPIRE faculty research grant.

ORCID

Himansu S. Biswal http://orcid.org/0000-0003-0791-2259
Konkallu Hanumae Gowd http://orcid.org/0000-0002-8991-9368

REFERENCES

1. Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature*. 2003;422(6928):198-207.
2. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF. Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proc Natl Acad Sci U S A*. 2004;101(26):9528-9533.
3. Gunawardena HP, Gorenstein L, Erickson DE, Xia Y, McLuckey SA. Electron transfer dissociation of multiply protonated and fixed charge disulfide linked polypeptides. *Int J Mass Spectrom*. 2007;265(2):130-138.
4. Mikesh LM, Ueberheide B, Chi A, et al. The utility of ETD mass spectrometry in proteomic analysis. *Biochim Biophys Acta*. 2006;1764(12):1811-1822.
5. Wiesner J, Premsler T, Sickmann A. Application of electron transfer dissociation (ETD) for the analysis of posttranslational modifications. *Proteomics*. 2008;8(21):4466-4483.
6. Wu S-L, Jiang H, Lu Q, Dai S, Hancock WS, Karger BL. Mass spectrometric determination of disulfide linkages in recombinant therapeutic proteins using online LC-MS with electron-transfer dissociation. *Anal Chem*. 2009;81(1):112-122.
7. Tureček F, Julian RR. Peptide radicals and cation radicals in the gas phase. *Chem Rev*. 2013;113(8):6691-6733.
8. Hopkinson AC. Radical cations of amino acids and peptides: Structures and stabilities. *Mass Spectrom Rev*. 2009;28(4):655-671.
9. Mentinova M, Han H, McLuckey SA. Dissociation of disulfide-intact somatostatin ions: the roles of ion type and dissociation method. *Rapid Commun Mass Spectrom*. 2009;23(17):2647-2655.
10. Cole SR, Ma X, Zhang X, Xia Y. Electron transfer dissociation (ETD) of peptides containing intrachain disulfide bonds. *J Am Soc Mass Spectrom*. 2012;23(2):310-320.
11. Clark DF, Go EP, Desaire H. Simple approach to assign disulfide connectivity using extracted ion chromatograms of electron transfer dissociation spectra. *Anal Chem*. 2013;85(2):1192-1199.
12. Wu S-L, Jiang H, Hancock WS, Karger BL. Identification of the unpaired cysteine status and complete mapping of the 17 disulfides of recombinant tissue plasminogen activator using LC-MS with

electron transfer dissociation/collision induced dissociation. *Anal Chem*. 2010;82(12):5296-5303.

13. Tan L, Durand KL, Ma X, Xia Y. Radical cascades in electron transfer dissociation (ETD) – implications for characterizing peptide disulfide regio-isomers. *Analyst*. 2013;138(22):6759-6765.
14. Kleinnijenhuis AJ, Duursma MC, Breukink E, Heeren RMA, Heck AJR. Localization of intramolecular monosulfide bridges in lantibiotics determined with electron capture induced dissociation. *Anal Chem*. 2003;75(13):3219-3225.
15. Lohans CT, Huang Z, van Belkum MJ, et al. Structural characterization of the highly cyclized lantibiotic paenicidin A via a partial desulfurization/reduction strategy. *J Am Chem Soc*. 2012;134(48):19540-19543.
16. Lohans CT, Vederas JC. Structural characterization of thioether-bridged bacteriocins. *J Antibiot (Tokyo)*. 2014;67(1):23-30.
17. Knerr PJ, van der Donk WA. Discovery, biosynthesis, and engineering of lantipeptides. *Annu Rev Biochem*. 2012;81:479-505.
18. Chrisman PA, Pitteri SJ, Hogan JM, McLuckey SA. $SO_2^{-\bullet}$ electron transfer ion/ion reactions with disulfide linked polypeptide ions. *J Am Soc Mass Spectrom* 2005;16(7):1020–1030.
19. Sobczyk M, Anusiewicz I, Berdys-Kochanska J, Sawicka A, Skurski P, Simons J. Coulomb-assisted dissociative electron attachment: Application to a model peptide. *J Phys Chem A*. 2005;109(1):250-258.
20. Syrstad EA, Turecček F. Toward a general mechanism of electron capture dissociation. *J Am Soc Mass Spectrom*. 2005;16(2):208-224.
21. Chen X, Turečekf F. The arginine anomaly: Arginine radicals are poor hydrogen atom donors in electron transfer induced dissociations. *J Am Chem Soc*. 2006;128(38):12520-12530.
22. Galande AK, Spatola AF. A facile method for the direct synthesis of lanthionine containing cyclic peptides. *Lett Pept Sci*. 2001;8(3):247-251.
23. Galande AK, Trent JO, Spatola AF. Understanding base-assisted desulfurization using a variety of disulfide-bridged peptides. *Biopolymers*. 2003;71(5):534-551.
24. Horn MJ, Jones DB, Ringel SJ. Isolation of a new sulfur-containing of a new amino acid(Lanthionine) from sodium carbonate-treated wool. *J Biol Chem*. 1941;138(1):141-149.
25. Horn MJ, Jones DB, Ringel SJ. Isolation of mesolanthionine from various alkali-treated proteins. *J Biol Chem*. 1942;144:87-91.
26. Thakur SS, Balaram P. Characterization of alkali induced formation of lanthionine, trisulfides, and tetrasulfides from peptide disulfides using negative ion mass spectrometry. *J Am Soc Mass Spectrom*. 2009;20(5):783-791.
27. Slootweg JC, Liskamp RMJ, Rijkers DTS. Scalable purification of the lantibiotic nisin and isolation of chemical/enzymatic cleavage fragments suitable for semi-synthesis. *J Pept Sci*. 2013;19(11):692-699.
28. Govindu PCV, Sudarshan C, Gowd KH. Synthesis of two closely spaced cysteine barbiturates containing peptides by copper-catalyzed oxidation of contryphan disulfide. *Synth Commun*. 2017;47(17):1559-1567.
29. Chu IK, Siu C-K, Lau JK-C, et al. Proposed nomenclature for peptide ion fragmentation. *Int J Mass Spectrom*. 2015;390(Supplement C):24-27.
30. Furche F, Ahlrichs R, Hättig C, Klopper W, Sierka M, Weigend F. Turbomole. *Wiley Interdiscip Rev Comput Mol Sci*. 2014;4(2):91-100.
31. Schäfer A, Horn H, Ahlrichs R. Fully optimized contracted Gaussian basis sets for atoms Li to Kr. *J Chem Phys*. 1992;97(4):2571-2577.
32. Schäfer A, Huber C, Ahlrichs R. Fully optimized contracted Gaussian basis sets of triple zeta valence quality for atoms Li to Kr. *J Chem Phys*. 1994;100(8):5829-5835.
33. Hao G, Gross SS. Electrospray tandem mass spectrometry analysis of S- and N-nitrosopeptides: facile loss of NO and radical-induced fragmentation. *J Am Soc Mass Spectrom*. 2006;17(12):1725-1730.
34. Breukink E, de Kruijff B. The lantibiotic nisin, a special case or not? *Biochim Biophys Acta*. 1999;1462(1–2):223-234.
35. Shin JM, Gwak JW, Kamarajan P, Fenno JC, Rickard AH, Kapila YL. Biomedical applications of nisin. *J Appl Microbiol*. 2016;120(6):1449-1465.
36. Inglis AS, Edman P. Mechanism of cyanogen bromide reaction with methionine in peptides and proteins. I. Formation of imidate and methyl thiocyanate. *Anal Biochem*. 1970;37(1):73-80.
37. Lee M, Lee Y, Kang M, et al. Disulfide bond cleavage in TEMPO-free radical initiated peptide sequencing mass spectrometry. *J Mass Spectrom*. 2011;46(8):830-839.
38. Zhang X, Julian RR. Exploring radical migration pathways in peptides with positional isomers, deuterium labeling, and molecular dynamics simulations. *J Am Soc Mass Spectrom*. 2013;24(4):524-533.
39. Leymarie N, Costello CE, O'Connor PB. Electron capture dissociation initiates a free radical reaction cascade. *J Am Chem Soc*. 2003;125(29):8949-8958.
40. Belyayev MA, Cournoyer JJ, Lin C, O'Connor PB. The effect of radical trap moieties on electron capture dissociation spectra of substance P. *J Am Soc Mass Spectrom*. 2006;17(10):1428-1436.
41. Moss CL, Chamot-Rooke J, Nicol E, et al. Assigning structures to gas-phase peptide cations and cation-radicals. An infrared multiphoton dissociation, ion mobility, electron transfer, and computational study of a histidine peptide ion. *J Phys Chem B*. 2012;116(10):3445-3456.
42. Biswal HS. Hydrogen bonds involving sulfur: New insights from ab initio calculations and gas phase laser spectroscopy. In: Scheiner S, ed. *Noncovalent Forces*. Cham: Springer International Publishing; 2015:15-45.
43. Mundlapati VR, Ghosh S, Bhattacherjee A, Tiwari P, Biswal HS. Critical assessment of the strength of hydrogen bonds between the sulfur atom of methionine/cysteine and backbone amides in proteins. *J Phys Chem Lett*. 2015;6(8):1385-1389.
44. Jakubowski JA, Sweedler JV. Sequencing and mass profiling highly modified conotoxins using global reduction/alkylation followed by mass spectrometry. *Anal Chem*. 2004;76(22):6541-6547.
45. Gowd KH, Dewan KK, Iengar P, Krishnan KS, Balaram P. Probing peptide libraries from Conus achatinus using mass spectrometry and cDNA sequencing: identification of delta and omega-conotoxins. *J Mass Spectrom*. 2008;43(6):791-805.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Dolle AB, Jagadeesh N, Bhaumik S, Prakash S, Biswal HS, Gowd KH. Electron transfer dissociation of synthetic and natural peptides containing lanthionine/methyllanthionine bridges. *Rapid Commun Mass Spectrom*. 2018;32:831-843. https://doi.org/10.1002/rcm.8108