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Studies of the degradation products of nisin, a peptide antibiotic, using capillary electrophoresis with off-line mass spectrometry

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Abstract

The utility of capillary electrophoresis (CE) for assessing the purity and stability of pharmaceutical peptides is investigated. The degradation of nisin, a pentacyclic peptide antibiotic, depends upon sample preparation and storage conditions and is followed by CE. With conventional UV detection, peaks are not identified and unresolved components are not detected. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOFMS) of isolated CE fractions provides molecular mass information that aids in identification of the nisin degradants and determination of peak purity. The purity of pure and degraded nisin in the absence of any separation is also determined using electrospray ionization mass spectrometry (ESI-MS) and MALDI-TOFMS.

Keywords: Capillary electrophoresis–mass spectrometry; Nisin; Peptides; Antibiotics

1. Introduction

The emerging technology of capillary electrophoresis (CE) has recently been investigated as a possible alternative to HPLC for drug analysis [1,2]. One particularly important area of drug analysis is the ability to separate and identify the degradants from a parent pharmaceutical compound. As the structures of the product compounds usually differ from the structure of the parent compound in only very subtle ways, this can be a challenging task. This is one advantage to the high separation efficiencies and peak capacities characteristic of CE. Numerous examples in the literature serve to illustrate the capability of CE to resolve chemical compounds possessing only minor differences in chemical structure [3–6].

Once separation conditions are optimized for a particular pharmaceutical compound, a CE electropherogram utilizing UV detection may sometimes reveal that more than one component is present. However, it is often advantageous to identify these components. In these instances, mass spectrometry becomes the ideal detector for CE, being capable of providing both molecular mass and structural information. Matrix-assisted laser desorption ionization (MALDI) in combination with time-of-flight mass spectrometry (TOFMS) has been shown to be a useful technique for the generation and analysis of molecular ions from biological molecules at the attomole to picomole level [7–9]. The off-line coupling of CE with MALDI-TOFMS for the analysis of proteins and peptides has been demonstrated recently [10]. Furthermore, electrospray ionization

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mass spectrometry (ESI-MS) has been employed to supply complementary, as well as confirmatory information regarding complex peptide mixtures [11]. In the present study, the purity and stability of the peptide pharmaceutical compound nisin is analyzed using high-performance capillary electrophoresis combined with off-line mass spectrometry.

Nisin is a peptide antibiotic that is produced by the bacterium *Lactococcus lactis*. It is mainly used as a food preservative in dairy products, and contains 34 amino acid residues with a relative molecular mass (M_r) of 3354.14 [12,13]. As illustrated in Table 1, eight of its residues are modified amino acids, including two dehydroalanines (ΔAla), one dehydrobutyrine (ΔAbu), one lanthionine (two alanines bonded to sulfur at the β-carbons, Ala_s–*S*–Ala_s), and four β-methyllanthionines, (D-Abu–*S*–Ala_s, where D-Abu is β-aminobutyric acid) giving rise to a series of cyclic units [12]. The structural integrity of the unusual residues plays a significant role in the biological function of this antibiotic, and the antimicrobial activity is lost or diminished upon modification or degradation of the dehydro groups [14]. Minor nisin is also a product of this bacterium, involving the substitution of the ΔAla residue of nisin at position 33 with a serine residue, and is expected to be present in all nisin samples.

Others have studied the chemical and physical properties of nisin, as well as its route of degradation [13–17]. Identification of a major component and two minor analogues in commercial nisin has been accomplished by ^{252}Cf plasma desorption mass

Table 1
Proposed structures of the known degradants of nisin (adapted from references [14,16,17])

Name	Calculated M_r	R_1	R_2
Major nisin	3354.1	H_2N–Ile–ΔAbu–D-Alas (ring A: Ile, ΔAla, Leu, Alas, S)	HOOC-Lys-ΔAla-Val-
Minor nisin [Ser^{33}] nisin	3372.2	H_2N–Ile–ΔAbu–D-Alas (ring A: Ile, ΔAla, Leu, Alas, S)	HOOC-Lys-Ser-Val-
[α-χ-Ala^5]-nisin	3372.2	H_2N–Ile–ΔAbu–D-Alas (ring A: Ile, CH_3, NH–C–C=O, OH, Leu, Alas, S)	HOOC-Lys-ΔAla-Val-
(*des*-ΔAla^5]-nisin	3372.2	H_2N–Ile–ΔAbu–D-Alas (Ile, NH_2, Pyr, Leu, Alas, S)	HOOC-Lys-ΔAla-Val-
Nisin$^{1-32}$	3156.9	H_2N–Ile–ΔAbu–D-Alas (ring A: Ile, ΔAla, Leu, Alas, S)	HOOC-Val-
(*des*-ΔAla^5)-nisin$^{1-32}$	3174.9	H_2N–Ile–ΔAbu–D-Alas (Ile, NH_2, Pyr, Leu, Alas, S)	HOOC-Val-

spectrometry [15]. A total of five degradants, reported independently by two research groups, has been identified in the literature, all isolated by HPLC [16,17]. The structures of the degradation products have been elucidated by nuclear magnetic resonance techniques and fast-atom bombardment mass spectrometry [12,14,16,17]. All degradations reported in the literature occur at either the carboxyl terminal of the peptide or in the A ring (identified in Table 1) of the molecule. In fact, all described degradations involve either one or both of the dehydroalanines at the 5 and 33 positions. Table 1 is a summary of each of the previously identified degradants, as well as their proposed structures.

Degradation of nisin can be initiated by light, pH, or temperature. Light induces free-radical reactions of the dehydro residues [13]. When treated in dilute acid at room temperature, nisin degrades at the ΔAla33 residue. When treated in dilute acid at elevated temperature (50°C), nisin degrades at both the ΔAla33 and ΔAla5 dehydro residues [16]. Above pH 8, nisin is unstable and becomes inactivated, with the products at high pH being mostly dimers and multimers [13]. Some of the known degradants are unstable and can further degrade. For example, the A ring of minor nisin can further degrade. Each of the degradants has been shown to possess differing levels of antimicrobial activity [13,14,16,17]. Developing an analytical method capable of monitoring the extent of nisin degradation with time is important for determining its efficacy as an antibiotic.

When using capillary electrophoresis to monitor these degradants, differences in the electrophoretic mobilities determine the selectivity of the separation [18]. For peptides that possess similar molecular masses and p*I*s, variation of the buffer pH is often the most direct route for optimizing the separation. A separation optimization strategy for the routine analysis of pharmaceutical peptides based upon pH has been proposed by Langenhuizen and Janssen [19]. They suggest that for peptides with a basic or neutral character, the best separation is achieved with low pH buffers. Further improvements in separation can be obtained by fine tuning the buffer pH and/or by using a higher buffer concentration. Additional improvements in the separation can be attempted through the use of various buffer additives. Pessi et al. [3] found that addition of organic modifiers such as methanol or acetonitrile to the separation buffer improved the separation of peptides that differed in only single, neutral amino acid residues. Addition of an organic modifier to a CE buffer substantially reduces the magnitude of the electroosmotic flow, leading to marked improvements in resolution [20].

Because nisin is a basic peptide, the strategy of Langenhuizen and Janssen indicates that a separation in a low-pH buffer is optimum. At low pH, protonation of the silanol groups of the capillary wall [21] can serve to minimize adsorption of nisin. The solubility characteristics of nisin also favors use of a low-pH separation buffer. Nisin is readily soluble in dilute acids and organic solvents such as methanol and acetonitrile, less soluble in water, and sparingly soluble in alkaline solution. Therefore, variation in the pH of the separation buffer, and use of an organic modifier additive to enhance selectivity is the main strategy that we use in the development and optimization of a CE separation of nisin from its degradants.

2. Experimental

2.1. Reagents and materials

Samples of "pure" and "degraded" nisin were from ConvaTec (St. Louis, MO, USA). The degraded nisin sample was of the same lot as the pure sample that had been incubated at 60°C for 1 year. Phosphoric acid (33 m*M*) was from Waters (Milford, MA, USA). Sodium phosphate monobasic, monohydrate and sodium phosphate, dibasic were from EM Science (Gibbstown, NJ, USA). Trifluoroacetic acid (TFA), 99%, formic acid, 96%, and α-cyano-4-hydroxycinnamic acid (ACHA), 97% were from Aldrich (Milwaukee, WI, USA). KOH (1 *M*), concentrated HCl, and Optima grade acetone, were from Fisher Scientific (Fair Lawn, NJ, USA). HPLC-grade acetonitrile was from Mallinckrodt (Paris, KY, USA), and bovine insulin was from Sigma (St. Louis, MO, USA). All capillary electrophoresis solutions were prepared with ultra-pure Milli-Q water (Millipore, Bedford, MA, USA). The solutions

were degassed by sonication and filtered through 0.2-μm syringe filters prior to use. All mass spectrometry solutions were prepared with water from a Nanopure ultra-pure water system (Barnstead, Dubuque, IA, USA).

2.2. Capillary electrophoresis

All separations of degradants were performed on a Beckman P/ACE 2100 CE unit, utilizing System Gold software with 200 nm UV detection. In all separations, electrokinetic injections were used. An untreated fused-silica capillary (75 μm I.D.×360 μm O.D., 70 cm to detector, 77 cm total length (Polymicro Technologies, Phoenix, AZ, USA) was used for this work. Both a low-pH and a high-pH separation were developed. For the low-pH separation, the run buffer was 33 m*M* phosphoric acid–acetonitrile (85:15) and the run voltage was +15 kV at ambient temperature. The sample was dissolved in 1 m*M* phosphoric acid, 15% acetonitrile at a concentration of 1 mg/ml. For the high-pH separation, the run buffer was acetonitrile–12.5 m*M* phosphate buffer pH 8.05 (70:30), and the run voltage was +29 kV at ambient temperature. The sample was dissolved in 0.28 m*M* phosphate buffer, pH 8.05, 77.5% acetonitrile at a concentration of 0.375 mg/ml. Once the samples were prepared, they were protected from light. For both separations, the conductivity of the sample solution was kept less than the conductivity of the running buffer, so that analyte stacking occurred [22].

2.3. CE fraction collection

Prior to CE fraction collection, the degraded nisin sample was desalted using a C_{18} Sep-Pak cartridge (Waters, Milford, MA, USA). The desalted sample was prepared (4 mg/ml) in 1 m*M* phosphoric acid containing 50% acetonitrile. The quantity of each component injected electrokinetically at 8 kV was calculated using the equation

$$Q_i = (v_i + v_{eo})C_i \pi r^2 t$$

where Q_i is the number of moles of component i, $v_i + v_{eo}$ are the combined zone velocities in cm/s of the component i and the electroosmotic flow respectively, C_i is the concentration of component i in mol/cm^3, r is the radius of the internal diameter of the capillary in cm, and t is the time of injection in seconds [23]. We estimate that for each CE run, approximately 12 pmol of total degraded nisin was electrokinetically injected onto the capillary. In order to obtain enough of the minor components for analysis by MALDI-TOFMS, the effluent from more than one CE run must be collected. Multiple CE runs were made until about 10 pmol of each component were collected. Reproducibility of CE migration times was enhanced by rinsing the capillary between each run for 2 min each at $1.38 \cdot 10^4$ Pa (20 p.s.i.) pressure with 0.1% TFA, water, 0.5 *M* KOH, water, and then running buffer.

Three components from the low-pH CE separation were collected by using a limited volume insert vial at the outlet end containing 5 μl of running buffer. The timing of when to begin and finish collecting each fraction was calculated using the equation

$$t_e = \frac{L_t}{L_d} t_d$$

where t_e is the time of elution from the capillary in minutes, L_t is the total capillary length in cm, L_d is the length of capillary to the detector in cm, and t_d is the observed migration time to the detector in minutes [24]. Compensation was made for the voltage ramp time when the outlet vial was switched to the collection vial. The voltage was decreased from 15 to 3 kV during the fraction collection period.

2.4. MALDI-TOFMS

Collected CE fractions and standards were prepared for subsequent MALDI-TOFMS according to the strategy recently developed by Vorm and coworkers [9,25]. Briefly, the matrix material (ACHA) was dissolved (5 mg/ml) in acetone that contained 2% water. Approximately 0.5 μl of matrix solution was spotted onto each MALDI target position by pipetting. A portion of the collected CE fraction, estimated to be about 1 pmol, was then deposited

onto the matrix surface. After the solvent had dried at ambient temperature, the samples were washed with 2 μl of 0.1% aqueous TFA.

MALDI-TOFMS analysis was performed on a Fisons/VG TofSpec mass spectrometer, operating at 18 kV accelerating potential for positive ions detected in the reflectron mode. Data acquisition and processing was controlled by a Fisons/VG OPUS data system running on a VAXstation 4000 computer. A nitrogen laser (337 nm, 5 ns pulse) was employed as the desorption/ionization source. The laser beam was focused at the sample surface with just enough energy to reach the ionization threshold. Each spectrum was generated by averaging signals from 30–60 laser pulses. Calibration was performed externally by using the known M_r values of insulin and matrix peaks. The M_r values and spectra were obtained by applying a smoothing function (one pass of five-point averaging) to the raw data.

2.5. ESI-MS

A Fisons/VG Quattro mass spectrometer, in combination with MassLynx software, was used for acquiring positive-ion ESI-MS spectra of pure and degraded nisin. Aqueous samples were diluted to a concentration of 100 pmol nisin/μl using water–acetonitrile (1:4) containing 0.1% aqueous formic acid. A total of 10 μl of this solution was injected. In addition to the ESI mass spectra, results were reported as mass distributions, as calculated by transformation of the raw spectra using MassLynx software.

3. Results and discussion

3.1. Capillary electrophoresis separation optimization

By considering the effect of buffer pH, ionic strength and organic modifier concentration on nisin solubility, two CE separations of nisin and its degradants are developed, one at low pH and one at high pH. For both separations, phosphate and acetonitrile are selected for running buffer components as these reagents exhibit low UV absorbance at the detection wavelength of 200 nm. UV detection of peptides is typically done in the range of 190–220 nm, exploiting the absorptivity of the carbonyl bonds in the peptides [18].

Looking at the results of the low pH separation in Fig. 1, even the pure nisin sample contains a number of trace components. An overlay of the pure and degraded electropherograms shows that the trace components in the pure sample are enhanced in the degraded sample. However, major nisin remains the main component, even in the degraded sample. Not all components are baseline resolved.

For the high-pH separation shown in Fig. 2, major nisin is not the component with the greatest peak height. Nisin undergoes more extensive degradation during the high-pH separation than during the low-pH separation. Again, baseline resolution of all components is not achieved.

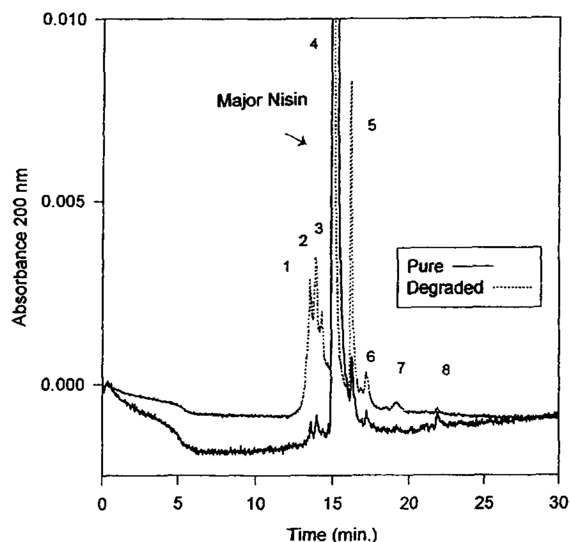

Fig. 1. Electropherograms of pure (enlarged) and degraded nisin, low-pH separation. Capillary, 75 μm I.D.×360 μm O.D. bare capillary with 77 cm total length. Run voltage, +15 kV. Run buffer, 33 mM phosphoric acid–acetonitrile (85:15). Sample, 1 mg/ml in 1 mM phosphoric acid, 15% acetonitrile. Injection, 5 s at 8 kV. Temperature, 25°C. The enlargement of the pure nisin electropherogram (not to scale) shows the presence of multiple trace components.

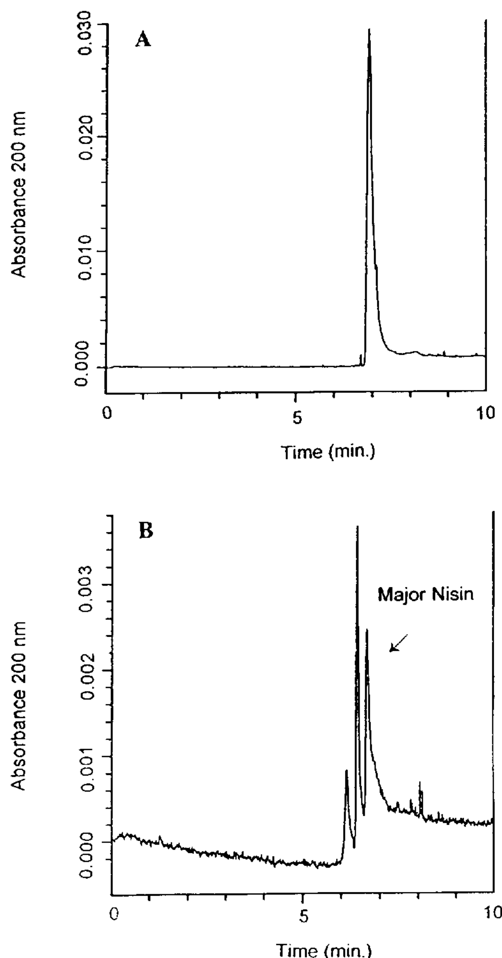

Fig. 2. (A) Electropherogram of pure nisin, high-pH separation, 0.4 mg/ml in 77.5% acetonitrile, 0.28 m*M* phosphate buffer, pH 8.05. (B) Electropherogram of degraded nisin, high-pH separation, 0.375 mg/ml in 77.5% acetonitrile, 0.28 m*M* phosphate buffer, pH 8.05. Capillary, 75 μm×360 μm O.D. bare capillary with 77 cm total length. Run voltage, +29 kV. Run buffer, acetonitrile–12.5 m*M* phosphate buffer pH 8.05 (70:30). Injection, 5 s at 8 kV. Temperature, 25°C.

Optimization of a separation involves gaining the maximum resolution of components in the least amount of time. More than seven components besides major nisin separate in approximately 23 min at low pH; the high-pH separation reveals only three other components besides major nisin, but in 8 min separation time. The high-pH separation is faster because the electroosmotic flow velocity is significantly greater at high pH than at low pH [26]. Whether all components are not resolved during the high-pH separation or whether the extent of degradation is accelerated due to the instability of nisin at high pH is not known. However, by looking more closely at the peak areas (not shown) from all of the nisin separations, we observe that the extent of total degradation of nisin is always greater in the samples run at high pH than for samples run at low pH, even when all samples are prepared freshly and run immediately after preparation. Therefore, how the nisin sample is prepared and what buffer it is dissolved in for analysis greatly affects not only the extent of degradation but also which degradant components are present.

This conclusion is verified by experiment. Fig. 1 and Fig. 3A, and 3B show electropherograms for nisin samples incubated at 60°C for 1 year, incubated in 1.0 *M* HCl at 60°C for 1.5 h, and incubated in 50 m*M* phosphate buffer pH 8 at 60°C for 1.5 h, respectively. All three electrophoretic profiles are different, even though the same low-pH separation is used. However, the high temperature, high-pH sample shows the greatest differences, again demonstrating the instability of nisin at basic pHs.

3.2. MALDI-TOFMS and ESI-MS of pure and degraded nisin

MALDI-TOFMS spectra are relatively easy to interpret, as the dominant peaks in the range of interest are composed of $[M+H]^+$ ions. $[M+2H]^{2+}$ ions are also observed in some cases. The matrix material is responsible for the abundance of peaks in the low M_r region of each spectrum. Interpretation of ESI-MS spectra, however, is generally more difficult, due to the production of ions with many charge states between $[M+3H]^{3+}$ and $[M+6H]^{6+}$.

Mass spectra of the nisin samples in absence of a separation provides information concerning purity and composition. The ESI-MS mass spectra of pure and degraded nisin are presented in Fig. 4A and 4B, respectively. Fig. 4C shows the mass distribution of both pure and degraded nisin samples, as transformed from the ESI-MS data, and Fig. 5 shows

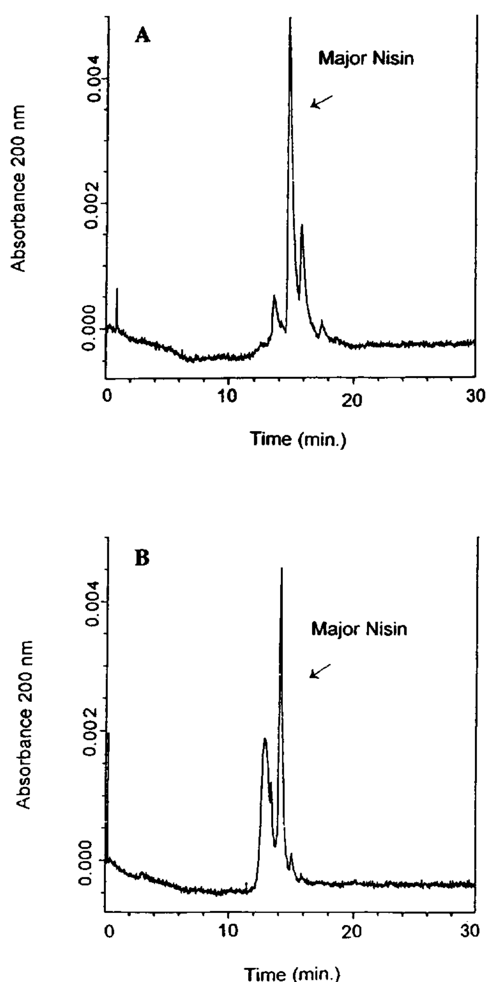

Fig. 3. (A) Electropherogram (using the low-pH separation) of pure nisin that had been incubated in 1.0 M HCl at 60°C for 1.5 h. Sample, 2 mg/ml of the treated nisin in 25% acetonitrile, 0.1 M HCl. (B) Electropherogram (using the low-pH separation) of pure nisin that had been incubated in 50 mM phosphate buffer, pH 8.05 at 60°C for 1.5 h. Sample, 0.25 mg/ml of the treated nisin in 50 mM phosphate buffer, pH 8.05.

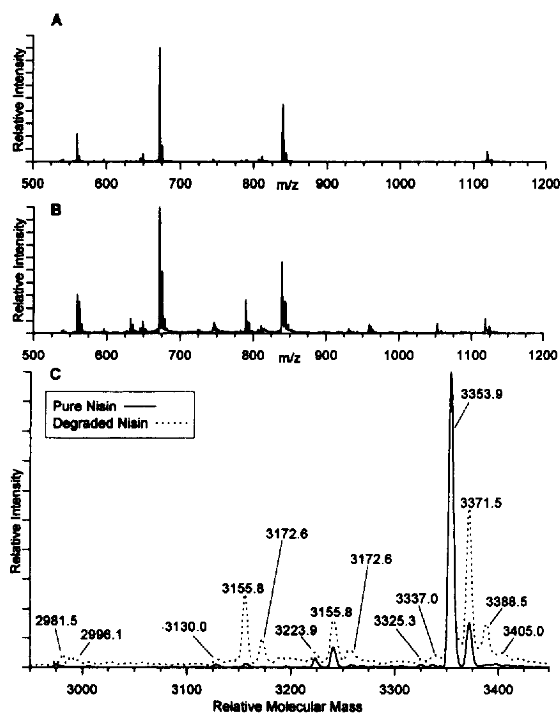

Fig. 4. (A) ESI-MS mass spectrum of pure nisin. (B) ESI-MS mass spectrum of degraded nisin. (C) Transformed ESI-MS mass distribution of pure and degraded nisin.

MALDI-TOFMS spectra of each nisin sample, where the inset shows the mass-to-charge range 0–4000 and the foreground focuses on the molecular-ion region. Major nisin, its five known degradation products, as well as some additional unidentified degradants or impurities are detected by both ionization techniques. The detected M_r values agree with the calculated values, within the estimated experimental error of 0.1% or 3 mass units.

3.3. MALDI-TOFMS of CE fractions

The order of migration of nisin and its degradants in the low-pH (pH 1.81) CE separation can be estimated using charge/size parameters, since peptide mobility is usually proportional to $z/m^{2/3}$, especially for a series of structurally similar degradants [18]. The charge on the nisin peptides is estimated by using the Henderson–Hasselbalch equation and adjusted pK_a values of amino acid residue functionalities that take into account the shifts in ionization constants that occur when amino acids are joined to form peptides [27]. From the calculation of $z/m^{2/3}$ for nisin and each of its degradants at pH 1.81, we expect that all components will not be resolved. As examples, nisin, minor nisin, [α-χ-

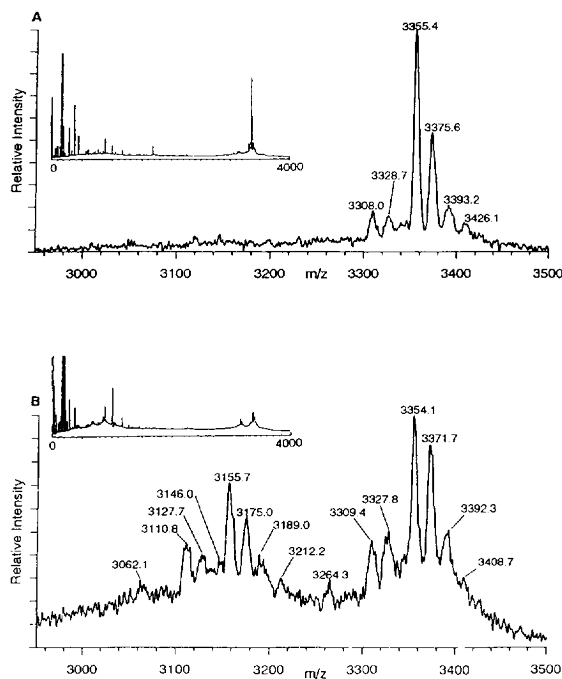

Fig. 5. Representative MALDI-TOFMS spectra of (A) pure and (B) degraded nisin. Each MALDI target spot contains approximately 0.5 pmol sample and 13 nmol of ACHA matrix.

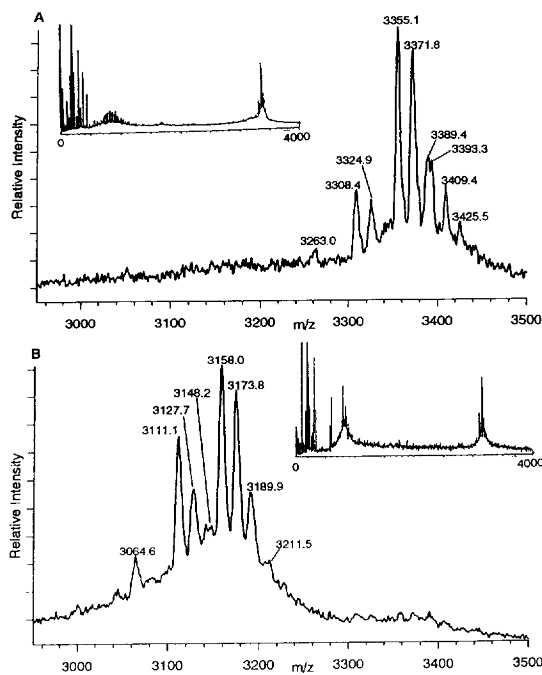

Fig. 6. Representative MALDI-TOFMS spectra of collected CE fractions: (A) peak 4 and (B) peak 5. Each MALDI target spot contains approximately 1 pmol sample and 13 nmol of ACHA matrix.

Ala^5]-nisin, and (*des*-ΔAla^5)-nisin may co-migrate as one peak, and nisin$^{1-32}$ and (*des*-ΔAla^5)-nisin$^{1-32}$ may co-migrate as another peak. As shown below, prediction is supported experimentally by fraction collection and MS.

To verify our ability to collect CE fractions, the major nisin peak of the low-pH pure nisin separation is collected, and a MALDI-TOFMS is obtained. The resulting spectrum (not shown) is virtually identical to the pure nisin spectrum shown in Fig. 5A.

The unresolved CE peaks labeled 1–3 of the low pH degraded nisin separation are collected and no M_r values are detected outside of the matrix region; thus peaks 1–3 appear to be small fragments. The two largest CE peaks labeled 4 and 5 are also collected with the mass spectra presented in Fig. 6. As predicted by the migration order calculations, peak 4 contains multiple species with M_r values corresponding to those of nisin, minor nisin, [α-χ-Ala^5]-nisin, and (*des*-ΔAla^5)-nisin. Because minor nisin, [α-χ-Ala^5] nisin and (*des*-ΔAla^5)-nisin have the same M_r value, it is not possible to discriminate between them. Peak 5 contains components corresponding to the M_r values of nisin$^{1-32}$ and (*des*-ΔAla^5)-nisin$^{1-32}$.

Several additional species are detected. These peaks may result from other degradants that are unresolved in the CE separation or degradants that have formed after the separation. These peaks may also arise from peak overlap due to inexact timing during fraction collection or from carryover between the outlet and collection vials. If the known nisin degradants further degrade, additional peaks would appear. For example, the M_r value detected by both MALDI-TOFMS and ESI-MS at 3390 may correspond to minor nisin that has undergone further degradation in the A ring. Table 2 lists the calculated M_r values of possible nisin degradants whose structures are in agreement to the experimentally measured M_r values shown in Fig. 4–6.

Table 2
Possible degradant structures of nisin that are in agreement with the experimentally determined M_r values

Calculated M_r	Possible degradant structure(s)	Ionization technique: Sample
2979	nisin$^{3-32}$ or minor nisin$^{3-32}$	ESI: Pure and degraded
2997	(*des*-ΔAla^5)-nisin$^{3-32}$ or (*des*-ΔAla^5)-minor nisin$^{3-32}$	ESI: Degraded
3065	nisin$^{3-33}$	MALDI: Degraded and CE peak 5
3113	nisin$^{2-33}$	MALDI: Degraded and CE peak 5
3131	(*des*-ΔAla^5)-nisin$^{2-33}$ or minor nisin$^{2-33}$	ESI: Pure and degraded, MALDI: Degraded and CE peak 5
3149	(*des*-ΔAla^5)-nisin$^{2-33}$+H_2O or (*des*-ΔAla^5)-minor nisin$^{2-33}$	MALDI: Degraded and CE peak 5
3157	nisin$^{1-32}$ or minor nisin$^{1-32}$	ESI: Pure and degraded, MALDI: Pure, degraded, and CE peak 5
3175	nisin$^{1-32}$+H_20 or (*des*-ΔAla$_5$)-nisin$^{1-32}$ or (*des*-ΔAla$_5$)-minor nisin$^{1-32}$	ESI: Degraded, MALDI: Pure, degraded, and CE peak 5
3193	nisin$^{3-34}$ or (*des*-ΔAla^5)-nisin$^{1-32}$+H_2O or (*des*-ΔAla$_5$)-minor nisin$^{1-32}$+H_2O	MALDI: Degraded and CE peak 5
3211	(*des*-ΔAla^5)-nisin$^{3-34}$	MALDI: Degraded and CE peak 5
3226	nisin$^{1-33}$	ESI: Pure and degraded
3241	nisin$^{2-34}$	ESI: Pure and degraded
3262	(*des*-ΔAla^5)-nisin$^{1-33}$+H_2O or (*des*-ΔAla^5)-minor nisin$^{1-33}$	MALDI: Degraded and CE peak 4
3373	minor nisin or nisin+H_2O or [α-χ-Ala^5]-nisin or (*des*-ΔAla^5)-nisin	ESI: Pure and degraded, MALDI: Pure, degraded, and CE peak 4
3390	(*des*-ΔAla^5)-nisin+H_2O or (*des*-ΔAla$_5$)-minor nisin	ESI: Degraded, MALDI: Pure, degraded and CE peak 4
3408	(*des*-ΔAla^5)-minor nisin+H_2O	ESI: Degraded, MALDI: Degraded and CE peak 4

4. Conclusions

The power of capillary electrophoresis with off-line mass spectrometry as a rapid separation/detection scheme in the evaluation of the purity and stability of pharmaceutical peptides is demonstrated. Two separations of a peptide antibiotic, nisin, and its degradants are developed, one at low pH and one at high pH. The low-pH separation reveals the presence of at least seven other components besides major nisin, the high-pH separation reveals three other components. Each analysis is complete in less than 25 min. The extent of degradation of nisin can be easily tracked by CE and is shown to depend on the sample preparation and storage conditions.

Five of the eight peaks separated by the low-pH method are collected from the capillary outlet and further evaluated by MALDI-TOFMS, with the smallest peaks having insufficient material for MS analysis. The results show that two of the collected fractions are not pure, but contain multiple species. The observed migration order agrees well with the predicted theoretical migration order based upon $z/m^{2/3}$ calculations. Using MALDI-TOFMS, all of the known nisin degradants, as well as nisin itself, can be accounted for in only two of the collected fractions. The inability of the selected optimized separation conditions to completely resolve all components is most likely due to the very similar mass-to-charge ratios of the degradation products.

MALDI-TOFMS and ESI-MS indicate the presence of M_r values that do not correspond to any of the known nisin degradants. However, additional work needs to be done in order to confirm the presence and identity of these unreported degradation products.

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References

[1] W. Steuer, I. Grant and F. Erni, J. Chromatogr., 507 (1990) 125–140.
[2] S. Fazio, R. Vivilecchia, L. Lesueur and J. Sheridan, J. Am. Biotechnol. Lab., 8 (1990) 10–17.
[3] A. Pessi, F. Bianchi, L. Chiappinelli, A. Nardi and S. Fanali, J. Chromatogr., 557 (1991) 307–313.
[4] A.J. Tomlinson, L.M. Benson, J.P. Landers, G.F. Scanlan, J. Fang, J.W. Gorrod and S. Naylor, J. Chromatogr., 621 (1993) 239–248.
[5] J. Liu, A.K. Cobb and M. Novotny, J. Chromatogr., 519 (1990) 189–197.
[6] J.P. Landers, R.P. Oda and M.D. Schuchard, Anal. Chem., 64 (1992) 2846–2851.
[7] F. Hillenkamp, M. Karas, R.C. Beavis and B.T. Chait, Anal. Chem., 63 (1991) 1193A–1203A.
[8] J.A. Castoro, R.W. Chiu, C.A. Monnig and C.L. Wilkins, J. Am. Chem. Soc., 114 (1992) 7571–7572.
[9] O. Vorm, P. Roepstorff and M. Mann, Anal. Chem., 66 (1994) 3281–3287.
[10] J. Cai and J. Henion, J. Chromatogr. A, 703 (1995) 667–692.
[11] J.R. Perkins, B. Smith, R.T. Gallagher, D.S. Jones, S.C. Davis, A.D. Hoffman and K.B. Tomer, J. Am. Soc. Mass Spectrom., 4 (1993) 670–684.
[12] M. Barber, G.J. Elliot, R.S. Bordoli, B.N. Green and B.W. Bycroft, Experientia, 44 (1988) 266–270.
[13] W. Liu and J.N. Hansen, Appl. Environ. Microbiol., 56 (1990) 2551–2558.
[14] L.Y. Lian, W.C. Chan, S.D. Morley, G.C.K. Roberts, B.W. Bycroft and D. Jackson, Biochem. J., 283 (1992) 413–420.
[15] P. Roepstorff, P.F. Nielsen, L. Kamensky, A.G. Craig and R. Self, Biomed Mass. Spectrom., 15 (1988) 305–310.
[16] W.C. Chan, B.W. Bycroft, L.Y. Lian and G.C.K. Roberts, FEBS Letters, 252 (1989) 29–36.
[17] H.S. Rollema, P. Both and R.J. Siegen, Proc. First Int. Workshop on Lantibiotics, 1991, pp. 123–130.
[18] R.M. McCormick, in J.P. Landers (Editor), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994, Ch. 12, pp. 287–323.
[19] M. Langenhuizen and P. Janssen, J. Chromatogr., 638 (1993) 311–318.
[20] K.D. Altria and C.F. Simpson, Anal. Proc., 23 (1986) 453–454.
[21] R.M. McCormick, Anal. Chem., 60 (1988) 2322–2328.
[22] D.S. Burgi and R.L. Chien, Anal. Chem., 63 (1991) 2042–2047.

[23] J. Harbaugh, M. Collette and H.E. Schwartz, Technical Bulletin TIBC-103, Beckman Instruments, Palo Alto, CA, 1990, pp. 1–2.
[24] R. Biehler and H.E. Schwartz, Technical Bulletin TIBC-105, Beckman Instruments, Palo Alto, CA, 1991, pp. 1–5.
[25] O. Vorm and M. Mann, J. Am. Soc. Mass Spectrom., 5 (1994) 955–958.
[26] B.B. Van Orman, G.G. Liversidge, G.L. McIntire, T.M. Olefirowicz and G. Ewing, J. Microcolumn Sep., 2 (1990) 176–180.
[27] B. Skoog and A. Wichman, Trends Anal. Chem., 5 (1986) 82–83.