^{15}N- and ^{13}C-Labeled Media from *Anabaena* sp. for Universal Isotopic Labeling of Bacteriocins: NMR Resonance Assignments of Leucocin A from *Leuconostoc gelidum* and Nisin A from *Lactococcus lactis*†

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ABSTRACT: A procedure for universal ^{13}C and/or ^{15}N labeling of microbial peptides which are produced by fermentation in complex media and its application to two food-preserving bacteriocins from lactic acid bacteria are described. Isotopic enrichment of nisin A (from *Lactococcus lactis*) and of leucocin A (from *Leuconostoc gelidum*) is readily achieved using a soluble peptone derived from enzymatic hydrolysis (pepsin and chymopapain) of *Anabaena* sp. ATCC 27899 cells grown on sodium [^{13}C]bicarbonate and/or sodium [^{15}N]nitrate as sole carbon and nitrogen sources. Combustion of this peptone followed by mass spectrometric analysis indicates that 45% of the labeled carbon and 65% of the labeled nitrogen added to the *Anabaena* culture are utilized in the amino acids of the peptone and that the isotopic purity for both ^{13}C and ^{15}N remains essentially unchanged provided that the cells are grown under argon atmosphere to avoid nitrogen fixation. NMR analyses of [^{13}C,^{15}N]nisin A using H{^{13}C}MQC, H{^{13}C}MBC, 2D INADEQUATE, and H{^{15}N}MQC techniques confirmed ^1H spectral assignments previously reported for unlabeled material and readily provided carbon and nitrogen assignments. The results show that universal *but not uniform* ^{13}C labeling occurs unless the nutrient source is completely isotopically enriched at high level (≥98%) because of differential levels of de novo amino acid synthesis. Application of NMR techniques such as TOCSY, DQF-COSY, NOESY, and H{^{13}C}MQC to unlabeled and [^{13}C]leucocin A afforded the complete ^1H and ^{13}C assignment. Leucocin A does not possess clearly defined conformational structure in DMSO or aqueous solutions.

Many recent NMR studies of the solution structure of peptides and proteins rely on isotopic labeling with ^{13}C and/or ^{15}N to overcome problems of overlap of ^1H resonances in 2D[1] homonuclear experiments and allow spectral assignment by heteronuclear multidimensional correlation techniques (e.g., HMQC, HMBC, HNCO, HNCA, etc.) [for leading references, see Clubb et al. (1992), Grzesiek and Bax (1992), MacKenzie et al. (1992), Powers et al. (1992), and Stockman et al. (1992)]. The resulting ^1H, ^{15}N, and ^{13}C spectral assignments provide a basis for investigations of conformation and dynamics of proteins in solution (Neri et al., 1992; Wagner et al., 1992; Bax, 1991) and also assist detailed analyses of intermolecular interactions such as binding to substrates, inhibitors, or receptors (Ikura & Bax, 1992; Fesik, 1991). However, the required isotopically substituted proteins bearing uniform ^{13}C and ^{15}N labels can be difficult and expensive to obtain. This is due to the cost of labeled [^{13}C,^{15}N]amino acids and [U-^{13}C]glucose, which are commonly used as precursors in fermentations (Henry & Sykes, 1992; Nicholson et al., 1992), as well as to the failure of many biological systems to produce desired metabolites on simple defined media. One solution is to generate the desired labeled protein directly from organisms such as cyanobacteria (blue green algae) which are capable of de novo synthesis of all required amino acids from minimal media (e.g., $^{13}CO_2$ and $Na^{15}NO_3$), as has been done for cytochromes (Stockman et al., 1989; Yu & Smith, 1988), ferredoxin (Oh et al., 1988), and for nonribosomally produced cyanobacterial and fungal peptides (Moore et al., 1989; Yee & O'Neil, 1992). A powerful alternative approach is cloning and overexpression of the encoding gene in *Escherichia coli*, where uniform labeling can be achieved by growth on [^{15}N]ammonium salts (sulfate or chloride) and/or [U-^{13}C]glucose (McIntosh & Dahlquist, 1990; Wang et al., 1992). However, apart from the cost of labeled glucose, which can sometimes be replaced by less expensive sodium [$^{13}C_2$]-acetate (Venters et al., 1991), this method is limited if the desired protein or peptide structure has undergone posttranslational modifications (amidation, glycosylation, isoprenylation, etc.). In such cases, or when the desired material cannot be easily generated in *E. coli*, the use of complex labeled media derived from hydrolysates (peptones) of yeast or bacterial cells grown on simple isotope-bearing precursors provides an attractive alternative for labeling of proteins (Kainosho et al., 1987; Westler et al., 1988; Zuiderweg & Fesik, 1989; Powers et al., 1992). Recently, ^{13}C and/or ^{15}N cell hydrolysates have become commercially available and some have been employed successfully for protein labeling (Powers et al., 1992), but they can fail to support growth of certain organisms or production of desired metabolites. This may be due to loss of essential components (e.g., chemically

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[1] Abbreviations: 2D, two dimensional; DQF-COSY, double-quantum filtered correlation spectroscopy; DMSO, dimethyl sulfoxide; GARP, globally optimized alternating-phase rectangular pulse; HMBC, heteronuclear multiple-bond correlation; HMQC, heteronuclear multiple-quantum correlation; INADEQUATE, incredible natural abundance double-quantum transfer; NOESY, nuclear Overhauser enhancement spectroscopy; TFA, trifluoroacetic acid; TOCSY, total correlation spectroscopy; TSP, (trimethylsilyl)propionic acid.

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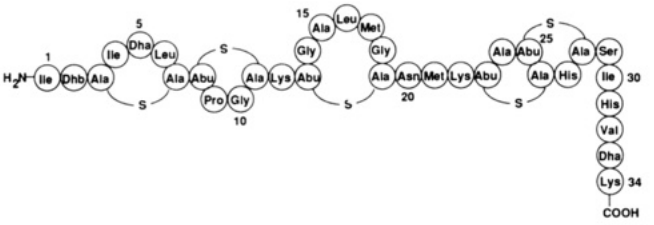

FIGURE 1: Structure of nisin A. Abbreviations for unusual amino acids are Dhb, 2-amino-2-butenoic acid (dehydrobutyrine); Dha, 2-amino-2-propenoic acid (dehydroalanine); Abu, 2-aminobutanoic acid.

labile amino acids) during processing (e.g., acid hydrolysis) or to their deficiency in the original cells. In the present work, we describe in detail a labeling technique based on the facile and inexpensive preparation of universally ^{13}C- and/or ^{15}N-labeled peptones derived from cyanobacterial cultures (*Anabaena* sp. ATCC 27899) grown on sodium [^{13}C[bicarbonate and sodium [^{15}N]nitrate as the sole carbon and nitrogen sources. The resulting labeled medium supports growth of a variety of microorganisms, including *E. coli*. We also report a combustion–mass spectrometric method for analysis of this peptone for isotopic content and use the labeling technique to assist the NMR assignment of two bacteriocins from lactic acid bacteria, nisin A from *Lactococcus lactis* ATCC 11454 (Figure 1) and leucocin A from *Leuconostoc gelidum* UAL 187.

Nisin A is bactericidal against a broad range of Gram-positive organisms and is approved for commercial use in over 45 countries as a nontoxic food preservation agent in dairy products (Delves-Broughton, 1990). Its unusual structure, which contains numerous sulfide bridges as a result of extensive posttranslational modifications, places it in the growing class of biologically active lantibiotics, peptides which contain lanthionine or β-methyllanthionine residues (Jung, 1991a,b; Schnell et al., 1992). The compound's commercial significance and interesting structural features, together with the lack of an X-ray crystal structure, have stimulated investigations using multidimensional NMR techniques on unlabeled samples which have afforded a complete proton assignment and information about possible solution conformations (Van de Ven et al., 1991; Lian et al., 1991, 1992; Goodman et al., 1991; Rollema et al., 1991).

Leucocin A belongs to an expanding class of nonlantibiotic bacteriocins (Klaenhammer, 1988; Joerger & Klaenhammer, 1990; Muriana & Klaenhammer, 1991; Hastings et al., 1991; Van Belkum et al., 1991; Holo et al., 1991) which have no posttranslational modifications (with the exception of disulfide bridges). Although they possess potential for use as food preservation agents for dairy products and meat, lack of information about the chemical structures and mechanism of action of these amphiphilic peptides has retarded their commercial development. We recently reported the genetic and amino acid sequence of leucocin A (Hastings et al., 1991) and described in preliminary form its ^1H and ^{13}C NMR assignment (Henkel et al., 1992) with the assistance of the labeling technique presented below.

EXPERIMENTAL PROCEDURES

Preparation of Labeled Peptone. A culture of the cyanobacterium *Anabaena* sp. ATCC 27899 served as a producer for the peptone in a modified BG-11 medium containing (per liter of solution): $MgSO_4 \cdot 7H_2O$, 75 mg; $CaCl_2 \cdot 2H_2O$, 50 mg; $K_2HPO_4 \cdot 3H_2O$, 40 mg; Sea Water Mix (Bio-Crystals Marinemix, Marine Enterprises, Baltimore, MD), 125 mg; ASMT

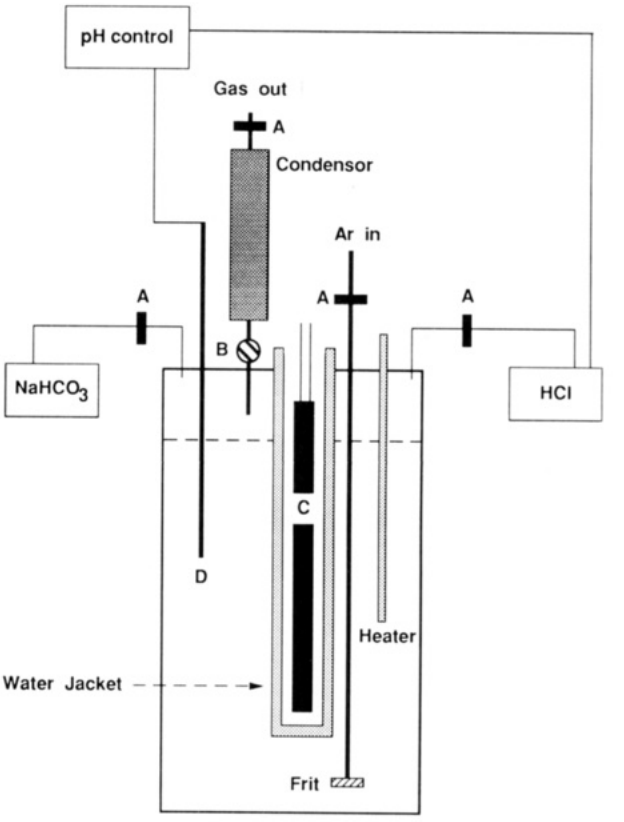

FIGURE 2: Apparatus for *Anabaena* sp. fermentation (10 L). All inlets and outlets for solutions and gases are protected by sterile filters (A). Effluent gas is monitored by an oxygen electrode (B). A filament light tube (C) provides light for photosynthesis together with banks of fluorescent lights surrounding the vessel (not shown). Temperature is controlled by a thermostated combination of an immersion heater in the medium and a cooling water jacket encasing the central light source. The medium is stirred continuously with a magnetic stirrer, and the sodium [^{13}C]bicarbonate solution is added by syringe pump. The 2 N HCl addition by peristaltic pump is controlled by a pH controller and monitored with a pH electrode (D).

Micro Elements Solution, 1 mL ($FeCl_3 \cdot 6H_2O$, 0.54 mg; Na_2-EDTA, 3 mg; H_3BO_3, 0.62 mg; $MnCl_2 \cdot 4H_2O$, 1.4 mg; $ZnCl_2$, 0.1 mg; MoO_3, 12 μg; $CoCl_2 \cdot 6H_2O$, 5 μg; $CuCl_2 \cdot 2H_2O$, 34 ng). Unlabeled sodium nitrate (550 mg/L) was added if only ^{13}C labeling was desired. The pH of the medium was adjusted to 7.6 prior to autoclaving at 121 °C for 20 min. The *Anabaena* sp. was maintained in 20-mL screw cap culture tubes (partially open to allow gas exchange) containing 10 mL of medium and reinoculated in fresh medium every 3 weeks. About 2 mL of the growing culture was transferred to a 500-mL Erlenmeyer flask with 100 mL of medium. The standing cultures were propagated at room temperature under cool white fluorescent tubes (photon flux density 20 μmol s^{-1} m^{-2} set to a photoperiod of 20 h on and 4 h off) for 12 days and then were used as an inoculum for large-scale fermentations. These employed 8 L of medium in a 10-L glass vessel equipped with magnetic stirrer, temperature controller, light source, pH controller, oxygen gas electrode, and fritted gas inlet (Figure 2). The pH of the gently stirred culture was maintained at 7.7 (± 0.1) by the addition of 2 N HCl using a pH controller, and the temperature was kept at 28 °C. The oxygen produced by *Anabaena* sp. photosynthesis was monitored with an oxygen gas electrode (Model 51B, Yellow Springs Instrument Co., Yellow Springs, OH) and was removed with a continuous flow of nitrogen (^{13}C-labeling experiments) or argon (^{15}N experiments). The sweep gas was purified using CO_2-

absorbing Ascarite (Arthur H. Thomas Co., Philadelphia, PA) and was bubbled through the culture such that concentration of oxygen did not exceed 5–10% in the gas leaving the system. For ^{13}C labeling, solutions of $NaH^{13}CO_3$ (50% or 99% isotopic purity; Cambridge Isotope Laboratories, Woburn, MA) in water were added continually with a syringe pump (60 mL per 24 h). The concentration of the bicarbonate solution was increased during the cultivation (1 g per 60 mL for days 1–2, 2 g per 60 mL for days 3–4, and 3 g per 60 mL for days 5–11). Nitrogen labeling was achieved by adding three 1.5-g portions of $Na^{15}NO_3$ (99% isotopic purity; Cambridge Isotope Laboratories), each dissolved in 10 mL of BG-11 medium after (a) autoclaving, (b) day 4, and (c) day 7 of cultivation. These aliquots were introduced to the media by injection through sterile syringe filters (0.2 μm). The nitrate level was tested by nitrate test strips (Merck, Darmstadt, Germany) and adjusted if the concentration in the culture dropped below 100 ppm to suppress nitrogen fixation by *Anabaena* sp. Growth of the culture was indicated by the HCl utilization. Good growth was established by supplying light from six cool white fluorescent tubes surrounding the vessel and one filament tube located in the interior (Figure 2). The intensity of light was gradually increased from 70 to 180 μmol s^{-1} m^{-2} total photon flux density over the first five days of the cultivation. The cultivations were stopped 2 days after reaching the stationary phase of growth (indicated by the ΔpH/min), for a usual total fermentation time of 10–11 days. The cells were collected by centrifugation (1500 *g*) at 5 °C and lyophilized. The typical yield was 0.8–1.0 g of dry cells per liter of fermentation. The dry cells were extracted with ethyl acetate in a Soxhlet extractor for 1 day to remove lipids, chlorophyll, and other pigments. The resulting blue residue was resuspended in water (1.5 g/100 mL) and then digested at 37 °C with pepsin (Sigma; 3300 units/mg) at a concentration of 70 units/mL for 12 h at pH 2.0 (adjusted with concentrated HCl). Subsequently, the pH was adjusted to 6.7 (with 2 N NaOH), and the mixture was incubated with chymopapain (Sigma; 4.5 units/mg) using 1 unit/mL for 36 h at 37 °C. The solution was autoclaved (121 °C, 20 min) and centrifuged (3000*g*) to remove insoluble components. The precipitate was extracted twice with water (300 mL) and centrifuged, and all supernatant fluids were combined before lyophilization. The typical yield was 0.65 g of peptone from 1 g of dry *Anabaena* cells.

Determination of ^{13}C and ^{13}N Enrichment. Samples of peptone (ca. 15 mg each) were combusted in an oxygen/helium atmosphere using a standard elemental analyzer (Perkin-Elmer, Model 240), and the gaseous products (mostly CO_2 and N_2 diluted with He) were collected in 150-mL glass containers designed to fit on a mass spectrometer (Kratos AEI MS12 instrument with single focusing magnetic sector and resolution of 1000). The isotopic enrichment was determined by comparison of the intensity of the observed peaks in the mass spectrum, namely, m/z 44 and 45 for $^{12}CO_2/^{13}CO_2$ and m/z 28, 29, and 30 for $^{14}N_2/^{14}N^{15}N/^{15}N_2$. Five determinations were each done in triplicate and compared with standards using unlabeled peptone.

Selection of Strain for Nisin A Production. L. lactis subsp. *lactis* ATCC 11454 was cultivated at 25 °C for 1–2 days in 5 mL of a medium (hereafter called LTB) containing (w/v) 1% meat extract (Difco), 1% yeast extract (Difco), 1% bactopeptone (Difco), 2.5% glucose, 0.5% NaCl, 0.2% $K_2HPO_4 \cdot 3H_2O$, and a vitamin mixture (nicotinic acid, 1 μg; pyridoxine·HCl, 1 μg; thiamine·HCl, 0.1 μg; vitamin B_{12}, 1 ng; folic acid, 1 ng; biotin, 1 ng; Ca panthothenate, 1 μg; riboflavine, 1 μg). The vitamin mixture was dissolved in 50 mM phosphate buffer (pH 7.0), sterilized by filtration, and added after autoclaving. The pH was adjusted to 6.8 with 1 N HCl prior to autoclaving. A high producing strain was selected by gradually adapting *L. lactis* subcultures on an increasing concentration of crude nisin A (500–10 000 units/mL; Aplin and Barrett, Trowbridge, Wiltshire U.K., 37 000 units/mg) added to the medium prior to inoculation. Highly resistant subcultures (identified by growth) were centrifuged, resuspended in 5 mL of fresh LTB medium, mixed with glycerol (4:1), and filled into sterile microfuge tubes in 0.5-mL portions. These cultures (stored at −60 °C) were the inoculum for all further experiments.

Production and Isolation of Nisin A. For generation of labeled nisin A, the extracts of the LTB medium were replaced with the labeled peptone (10 g/L), and the glucose concentration was increased (30 g/L). The fermentations typically employed 400 mL of labeled medium at 28–30 °C under a nitrogen atmosphere and were inoculated with a preculture (40 mL) grown in the same medium for 18 h at 26 °C. The pH was maintained at 6.8–6.9 by adding 1.5 N NaOH. After 22 h, the mixture was cooled to 4 °C and centrifuged (6000*g*; 20 min) to collect the cells. The cells were disrupted in 80 mL of 4 mM phosphate buffer (pH 7.2) at 4 °C with a bead beater (125 mL, Biospec Products, Bartlesville, OK) using 40 mL of 0.5-mm glass beads. After centrifugation (8000*g*; 20 min), the precipitate was extracted with 50 mL of HCl (50 mM) for 16 h at 4 °C and then again with 30 mL. The combined extracts were chromatographed on reverse-phase silica gel [C_{18} ChromSep; Chromatographic Specialities; equilibrated with 0.1% trifluoroacetic acid (TFA)] using 20%, 34%, and 60% MeCN/water/0.1% TFA. The 34% fraction was concentrated, lyophilized, dissolved in 20% MeCN/0.1% TFA (3 mL), and purified by HPLC (Waters μ-Bondapack C_{18}; 8 × 100 mm; 125 Å, 10 μm) using isocratic elution with 34% MeCN/0.15% TFA (flow rate 1.65 mL/min, monitored at 220 nm, 10 min). All nisin A samples were lyophilized and stored at −20 °C.

Production and Isolation of Leucocin A. The procedures previously reported (Hastings et al., 1991) for fermentation of *L. gelidum* UAL 187 and isolation of unlabeled leucocin A were modified. The medium for production of ^{13}C-labeled leucocin A contained per liter: ^{13}C-labeled peptone, 10 g; D-glucose, 25 g; $K_2HPO_4 \cdot 3H_2O$, 1 g; $MgSO_4$, 0.1 g; $MnSO_4 \cdot 4H_2O$, 0.05 g; and Tween 80, 1 mL. An inoculum (20 mL) of a 12 h old culture of *L. gelidum* was added to 1.7 L of the labeled medium. The culture was stirred under N_2 at 24 °C, and the pH was maintained at 6.0 by addition of 1 N NaOH (total 160 mL). After 32 h, the fermentation was stopped, and the mixture was centrifuged (7000*g*, 20 min, 4 °C) to separate the cells. The supernatant was purified on an Amberlite XAD-2 column (6 × 25 cm; preequilibrated with 0.1% TFA in water) by sequential elution with the following solvents containing 0.1% TFA: water (2 L); 20% EtOH (2 L); 40% EtOH (2 L); 70% EtOH (2 L); 85% EtOH (1 L). The combined active fractions (70–85% EtOH) were concentrated to 10 mL and applied to a Sephadex LH-60 column (2.5 × 45 cm) equilibrated with 50% MeCN/0.1% TFA. The antimicrobially active fractions were pooled and concentrated to 5 mL. The final purification of labeled leucocin A was accomplished by RP-HPLC with a Waters Deltapak-C_{18} column (8 × 100 mm, 10 μm, 300 Å, monitored at 220 nm, flow rate of 2 mL/min) in two steps using first a gradient from 30% to 35% MeCN/0.1% TFA and subse-

quently an isocratic elution with 30% MeCN/0.1% TFA. A total of 1.7 mg of labeled leucocin A was obtained.

NMR Sample Preparation. Nisin A samples were dissolved in either 90% H_2O/10% D_2O or 99.95% D_2O, resulting in peptide concentrations of 4 mM (unlabeled; ^{13}C-labeled) or 3 mM ($^{15}N/^{13}C$ labeled), respectively. The pH was adjusted to 2.1 by adding TFA (0.15%). Leucocin A (8 mg of unlabeled and 1.7 mg of ^{13}C-labeled) were each dissolved in 400 μL of DMSO-d_6 under a N_2 atmosphere, giving a total concentration of about 5 and 1 mM, respectively. Unlabeled leucocin A was also measured in a mixture of 400 μL of H_2O, 50 μL of D_2O, and 50 μL of DMSO-d_6 (4 mM). Both samples were acidified by adding 1 μL of TFA to enhance the stability. Field homogeneity during NMR measurements was improved by using matched susceptibility plugs (Varian Associates, Palo Alto, CA).

NMR Spectra. The nisin A spectra were recorded at 301 K on Varian Unity 500 and GN Omega 500 General Electric spectrometers with 5-mm probes. Leucocin A spectra were recorded on a Varian Unity 500 spectrometer at 283 and 298 K (H_2O samples) or at 283, 303, and 313 K (DMSO samples). Phase-sensitive two-dimensional spectra were obtained by using both the time-proportional phase incrementation method (Marion & Wüthrich, 1983) and the hypercomplex method (States et al., 1982). ^1H and ^{13}C chemical shifts were referenced to internal TSP. ^{15}N chemical shifts were determined relative to external benzamide, which was assigned a chemical shift of 105.4 ppm with reference to liquid ammonia. The signal of the water resonance line was usually suppressed by low-power irradiation during the relaxation delay and also during the mixing time of the NOESY experiment. Homonuclear NOESY (Jeener et al., 1979), clean-TOCSY (Griesinger et al., 1988), and DQF-COSY (Piantini et al., 1982; Shaka & Freeman, 1983) spectra were recorded with 256, 300, and 400 complex t_1 points, respectively, each with 2048 complex t_2 data points, 16 or 32 scans per t_1 increment, and a spectral width of 5500 Hz. For nisin A, mixing times of 300 (NOESY) and 65 ms (TOCSY) were used; the relaxation delay was typically between 1.6 and 1.8 s. The mixing times for NOESY spectra of leucocin A in water and DMSO were 300 and 400 ms or 250, 300, and 350 ms, respectively. The H{^{13}C}MQC (Bax & Subramanian, 1986) and H{^{13}C}MBC (Bax & Summers, 1986) spectra were recorded with 512 complex t_1 and 2048 complex t_2 data points applying 64 and 128 scans per increment, respectively, at natural abundance. The sweep width was 4400 Hz in F_2 and either 16 700 (HMQC) or 22 700 (HMBC) Hz in F_1. The nisin HMQC experiment was optimized for $^1J_{C,H}$ = 140 Hz, and a GARP pulse scheme was used for ^{13}C decoupling during the acquisition. The leucocin HMQC spectra were measured with 512 increments and 2K data points using a spectral width of 18 000 Hz in F_1 and 3700 Hz in F_2. For the unlabeled sample, 256 scans per increment were collected, for the labeled sample 32. Prior to Fourier transformation, all FIDs of leucocin proton data were multiplied by sine bell window functions shifted by $\pi/8$ or $\pi/16$ and zero-filled in F_1 to a data size of 2K by 2K. Delays for the HMBC were set for $^1J_{C,H}$ = 142 Hz and $^nJ_{C,H}$ = 5 Hz. The relaxation delay was varied between 0.8 and 1 s. For the H{^{15}N}MQC spectrum, 128 complex t_1 and 2048 complex t_2 data points were acquired with 16 scans per increment and a spectral width of 5500 Hz in F_2 and 2700 Hz in F_1 using the $^{15}N/^{13}C$-labeled sample. Only ^{15}N but not ^{13}C frequencies were decoupled with a WALTZ-16 pulse scheme during the acquisition. The relaxation delay was set to 1.4 s, and delays were optimized for a $^1J_{N,H}$ coupling of 90 Hz. The 2D INADEQUATE (Levitt & Ernst, 1983) spectrum was recorded with 256 complex t_1 and 4096 complex t_2 data points applying 256 scans per increment using the ^{13}C-labeled sample. A relaxation delay of 1.4 s was used, and the sequence was optimized for $^1J_{C,C}$ coupling of 40 Hz. To obtain a higher resolution, the spectrum was folded once in F_1 by setting the spectral widths in both F_1 and F_2 to 22 700 Hz. To investigate the level of carbon and nitrogen enrichment, ^{13}C and ^{15}N satellite intensities were observed in the 1D ^1H NMR spectra of both labeled nisin A samples. Data were collected on an inverse probe by using the transmitter channel to suppress the water resonance during the relaxation delay and, if necessary, the decoupler channel for the decoupling of either the ^{13}C or ^{15}N (WALTZ-16 scheme) nuclei during the acquisition. Prior to Fourier transformation, all FIDs were zero-filled in F_1 and multiplied by different window functions to gain the appropriate signal to noise ratio as well as resolution. The data were transformed on a Sun SPARC 2 workstation using either VNMR software (Varian Associates) or the FELIX (Hare Research, Bothwell, WA) program.

Table I: Amino Acid Composition of *Anabaena* Peptone[a]

amino acid	%	amino acid	%	amino acid	%	amino acid	%
Ala	10.4	Gly	9.49	Met	2.09	Thr	5.42
Arg	5.10	His	1.01	Orn	0.95	Trp	ND[d]
Asx[b]	10.8	Ile	5.54	Phe	3.79	Tyr	3.41
Cys[c]	0.97	Leu	8.72	Pro	4.88	Val	6.79
Glx[b]	11.0	Lys	4.75	Ser	4.88		

[a] Determined after complete acid hydrolysis. [b] The combined amounts of Asp and Asn as well as of Glu and Gln are given. [c] Separately determined as cysteic acid after acid hydrolysis in the presence of DMSO. [d] Tryptophan is destroyed by the analytical procedure and was not determined. Its presence was ignored in determining percent composition.

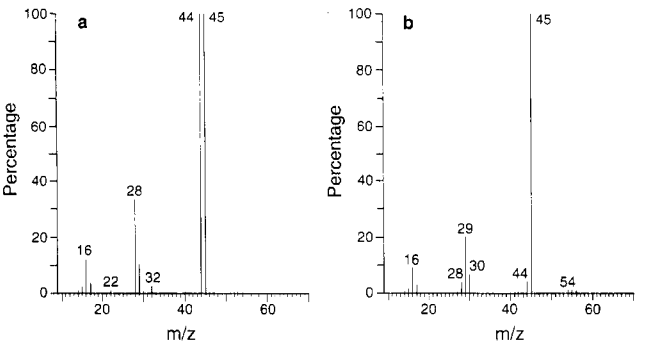

FIGURE 3: Mass spectra of gaseous products from elemental analyzer combustion of (a) [^{13}C]peptone from sodium [^{13}C]bicarbonate (50% isotopic purity) and (b) [^{13}C,^{15}N]peptone from sodium [^{13}C]-bicarbonate (99%) and [^{15}N]nitrate (99%). Isotope enrichment is calculated from peaks for CO_2 at m/z 44 and 45 and from peaks for N_2 at m/z 28, 29, and 30 by comparison to unlabeled standards. The peak at m/z 28 appears high in the ^{15}N-labeled sample because of the background of unlabeled atmospheric N_2 in the mass spectrometer.

RESULTS AND DISCUSSION

Production and Analysis of Isotopically Labeled Peptones. Although *Anabaena* sp. are easily grown, careful control of fermentation conditions enhances the yield of cells and the level of isotopic enrichment. Typical yields of dry *Anabaena* cells range from 0.8 to 1.0 per liter of fermentation medium and are now twice as high as reported earlier (Helms & Vederas, 1990). Extraction with ethyl acetate removes about 15% of the mass (pigments and lipids) and improves the quality of the final product as a medium for bacteriocin production.

Table II: ^{13}C and ^{15}N Chemical Shifts of Nisin A at 301 K in Water (0.2% TFA, pH 2.1)[a]

	^{15}N	C=O	Cα	Cβ	Cγ	Cδ	Cε
Ile 1		170.58	59.58	38.15	25.67, 16.11	12.34	
Δ^zAbu 2	125.5	168.07	130.00	134.75	14.37		
D-Ala$_s$ 3	117.3	173.70	55.98	35.67			
Ile 4	120.0	174.24	60.12	37.02	26.00, 16.99	11.90	
ΔAla 5	131.2	169.80	137.35	112.33			
Leu 6	125.4	174.73	54.68	38.67	26.22	22.78, 23.68	
Ala$_s$ 7	123.4	173.70	55.85	36.06			
D-Abu 8	117.4	170.58	59.38	51.05	23.12		
Pro 9		176.38	65.64	30.29	27.63	50.41	
Gly 10	106.6	173.27	43.80				
Ala$_s$ 11	121.7	174.86	55.33	38.15			
Lys 12	124.1, ε(140.3)	176.81	56.21	27.95	23.76	31.56	40.94
D-Abu 13	118.5	174.52	60.12	44.54	20.41		
Gly 14	111.8	173.11	44.05				
Ala 15	125.4	178.98	53.21	18.09			
Leu 16	118.9	176.97	54.79	40.51	26.22	22.08, 23.85	
Met 17	117.9	176.07	53.96	31.56	31.49		16.00
Gly 18	109.1	173.68	44.81				
Ala$_s$ 19	118.8	173.48	55.69	34.85			
Asn 20	112.0, γ(113.8)	173.71	52.33	37.84	175.97		
Met 21	122.2	175.72	54.17	31.70	30.97		15.93
Lys 22	125.0, ε(140.3)	176.81	56.21	27.95	23.85	31.56	40.94
D-Abu 23	118.6	174.59	60.18	49.58	22.15		
Ala 24	131.1	178.11	51.59	16.49			
D-Abu 25	120.3	174.72	61.50	46.36	21.80		
Ala$_s$ 26	123.9	173.80	57.61	38.96			
His 27	119.1	172.85	52.95	26.87	130.27	118.92	135.13
Ala$_s$ 28	121.8	173.44	54.27	38.99			
Ser 29	117.9	172.92	57.10	62.55			
Ile 30	123.3	173.12	59.94	37.88	26.06, 16.35	11.80	
His 31	124.2	172.85	54.01	27.95	129.74	119.00	135.27
Val 32	124.8	173.77	61.38	31.92	19.27, 20.00		
ΔAla 33	131.8	168.07	136.65	113.67			
Lys 34	120.8, ε(140.3)	177.98	54.27	27.83	23.92	31.98	40.94

[a] Shifts are given in parts per million with respect to TSP (0 ppm) for ^1H and benzamide (105.4 ppm) for ^{15}N (external).

The yield of soluble peptone obtained after enzymatic hydrolysis by commercially available pepsin and chymopapain is about 0.65 g from 1.0 g of dry cells (before extraction). The final peptone contains about 4% sodium chloride due to the preparation procedure. Its ability to act as a nutrient source depends on the nitrogen to carbon weight ratio (N:C). Elemental analysis of the dry cells (e.g., C, 42.04%; N, 8.88%; H, 5.99%; S, 1.17%) and peptone (e.g., C, 43.3%; N, 11.2%; H, 6.2%; S, 0.9%) gives N:C values of 0.21 (cells) and 0.28 (peptone), which are comparable to those of other complex media which are commonly employed as amino acid sources. Examples of the latter include meat extract (N:C 0.32), bactopeptone (N:C 0.35), and yeast extract (N:C 0.27). The amount of sulfur in the cyanobacterial peptone, though low, is about twice as high as normally found in standard commercial peptones. This can be advantageous for production of peptides or proteins with high sulfur content, such as nisin A and other lantibiotics. The amino acid composition of the *Anabaena* peptone, determined after complete acid hydrolysis, is given in Table I.

In order to determine efficiency of labeling, *Anabaena* peptones were examined by combustion in an elemental analyzer followed by mass spectrometry of the resulting gaseous products (predominantly CO_2 and N_2) (Figure 3). Comparison of peak intensities (five measurements each) at m/z 44 and 45 (CO_2) and 28, 29, and 30 (N_2) allows calculation of the various isotopic enrichments (Yamamoto & McCloskey, 1977). The material originating from fermentation under unlabeled nitrogen atmosphere using a mixture of sodium [^{15}N]nitrate and [^{13}C]bicarbonate (both 99% isotopic purity) or a mixture sodium [^{13}C]bicarbonate (50%) with unlabeled sodium nitrate shows enrichments of 97 ± 0.1% ^{13}C and 74.6 ± 4.9% ^{15}N for the [^{13}C,^{15}N]sample and 50 ± 0.1% ^{13}C for the [^{13}C]peptone. The greater standard deviation for the nitrogen enrichment is due to a slight variable contamination of peak intensities at m/z 29 and 28 by ^{13}C-labeled and unlabeled carbon monoxide which is formed because of incomplete sample combustion to carbon dioxide. The low background of atmospheric nitrogen in the mass spectrometer also contributes to the deviation. Despite the high labeled nitrate concentration, the ^{15}N enrichment was only about 75%, apparently due to fixation of the atmospheric N_2. This was surprising because the number of nitrogen-fixing cells (heterocysts, observed by microscope) was reduced to 10% of normal, and exclusive ^{15}N uptake from nitrate had been reported if its concentration exceeds 50 ppm (Bothe, 1982). This label dilution can be avoided by fermentation of *Anabaena* under argon atmosphere. Thus, growth of the cyanobacterial culture under an argon atmosphere with sodium [^{15}N]nitrate (3.0 g, 99% isotopic purity) and unlabeled sodium bicarbonate gave a peptone product whose combustion–mass spectrometric analysis shows 95.5 ± 2.6% ^{15}N. The expected level of ^{15}N labeling is close to 95% because unlabeled media (160 mL containing 0.11 g of unlabeled sodium nitrate) was added in the inoculum to an 8-L fermentation. If necessary, the isotopic enrichment could be increased by employing labeled media for the preculture and/or more labeled nitrate.

The results show that the isotopic purity of the universally labeled peptones is very close to that of the administered precursors, sodium bicarbonate, and/or nitrate, provided that the growth of the cyanobacteria proceeds under argon rather than nitrogen atmosphere. On the basis of the overall yields and isotopic analyses, 45% of the labeled carbon from sodium [^{13}C]bicarbonate is utilized in the universally labeled amino acids of the *Anabaena* derived peptone. For sodium [^{15}N]-nitrate, the corresponding efficiency of nitrogen conversion is

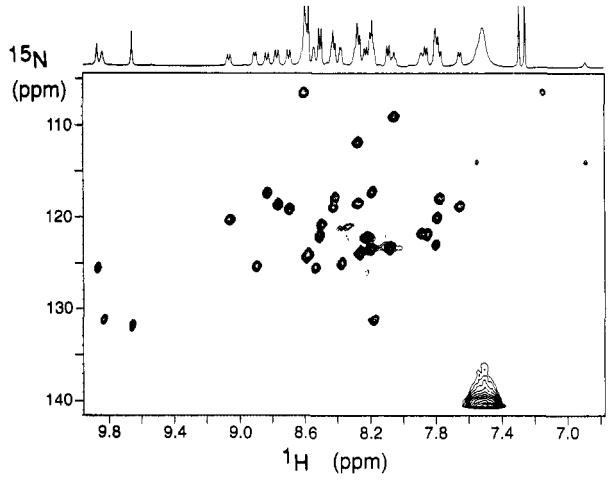

FIGURE 4: H{^{15}N}MQC spectrum of [^{13}C,^{15}N]nisin A (3 mM) dissolved in 9:1 H_2O/D_2O with 0.15% TFA. During acquisition, ^{15}N broad-band decoupling was applied, but carbons were not irradiated. The corresponding section (NH) of the one-dimensional ^{1}H NMR spectrum of unlabeled nisin A is shown on top for comparison.

65%. Before processing, the dry cells have an overall efficiency of label conversion of 60% for ^{13}C and 75% for ^{15}N. Since both of the precursors are relatively inexpensive, the present method provides a practical approach to obtaining a universally ^{13}C,^{15}N-labeled complex medium at substantially less cost than commercially available labeled cell hydrolysates. This peptone supports growth of many microorganisms, including *E. coli*, without supplementation by other nitrogen and/or amino acid sources. The *Anabaena* peptone may also prove useful for studies in animal systems with universally labeled algal protein. In a recent report on nutritional amino acid requirements and utilization in laying hens, the required labeled cyanobacterial (*Spirulina platensis*) protein was generated by closed system fermentation with $^{13}CO_2$ gas, which is considerably more difficult to handle (Berthold et al., 1991). In addition, the labeled *Anabaena* cells could also be a source for isotopically enriched DNA, RNA, and lipids.

Labeling of Nisin A and Its NMR Spectra. Since lantibiotics such as nisin A are products of extensive post-translational modification (Jung, 1991a,b; Schnell et al., 1992), standard genetic or amino acid sequencing techniques cannot by themselves provide complete primary structures (Piard et al., 1992; Stoffels et al., 1992). For such compounds, NMR spectroscopy can greatly assist structure elucidation and determination of possible solution conformations. After initiation of the present work (Helms & Vederas, 1990), ^{1}H NMR assignments have been reported for a number of unlabeled lantibiotics, including nisin A (Van de Ven et al., 1991; Lian et al., 1991, 1992; Goodman et al., 1991; Rollema et al., 1991), cinnamycin (Kessler et al., 1991), gallidermin (Freund et al., 1991a), pep5 (Freund et al., 1991b), and subtilin (Chan et al., 1992). Typical concentrations required in such experiments with unlabeled lantibiotics, which have molecular weights under 4000, range from 2 to 14 mM. The structures of a large number of bacteriocins remain unknown due to lack of sufficient quantities of material (Piard et al., 1992; Stoffels et al., 1992). Since universal labeling with ^{13}C and ^{15}N greatly enhances both the sensitivity and scope of NMR techniques, one goal of this study was to determine whether such labeling of bacteriocins from lactic acid bacteria can be achieved, in particular with the readily available *Anabaena* peptone.

Nisin A production by *L. lactis* was first increased 10-fold (up to 60 mg per liter of culture) by selection of nisin-resistant

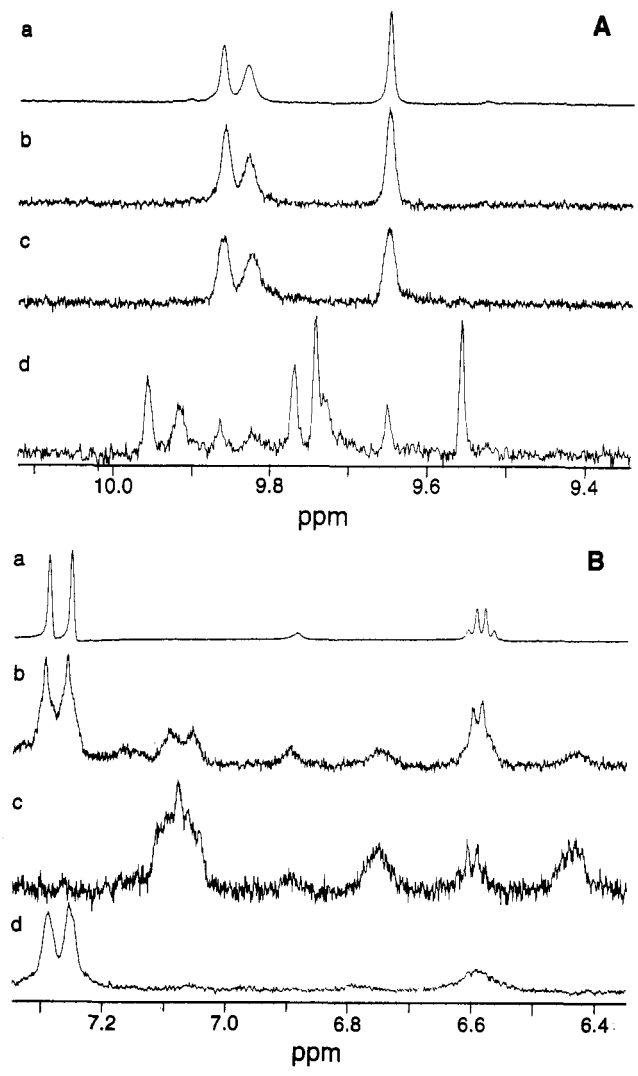

FIGURE 5: Expansions of ^{1}H NMR spectra of nisin A showing (A) NH resonances of residues 2, 5, and 33 (9.5–10.0 ppm) and (B) both histidine δ hydrogens (7.2–7.3 ppm) as well as the β-hydrogen of residue 2 (6.59 ppm). The individual spectra refer to (a) unlabeled nisin A, (b) [^{13}C]nisin A, (c) [^{13}C,^{15}N]nisin A with ^{15}N broad-band decoupling, and (d) [^{13}C,^{15}N]nisin A with ^{13}C broad-band decoupling. Multiplets appear broad due to incomplete WALTZ-16 ^{13}C decoupling and sample heating. Chemical shifts of isotopically shifted peaks are corrected to values of unlabeled nisin A for clarity.

strains. Subsequent cultivations of this organism on the [^{13}C]-peptone (50 ± 0.1% isotopic purity) and on the doubly labeled [^{13}C,^{15}N]medium (97% ^{13}C; 75% ^{15}N) followed by extraction of only the cells and purification afforded 5 mg of [^{13}C]nisin A and 3 mg [^{13}C, ^{15}N]nisin A, respectively. Examination of the DQF-COSY (Piantini et al., 1982; Shaka et al., 1983), TOCSY (Davis & Bax, 1985; Griesinger et al., 1988), and NOESY (Jeener et al., 1979) spectra by well-established methodology (Wüthrich, 1986) reconfirmed published ^{1}H NMR data for unlabeled nisin A (Van de Ven et al., 1991; Lian et al., 1991, 1992; Goodman et al., 1991; Rollema et al., 1991) for our slightly different solvent and temperature conditions. The chemical shift assignment of the carbons (Table II), which had not been previously reported, is available from the H{^{13}C}MQC, H{^{13}C}MBC, and INADEQUATE spectra of the U-^{13}C-labeled sample. From the INADEQUATE spectra, all of the expected ^{13}C–^{13}C interactions including those to quaternary carbons can be identified, thereby giving the full carbon connectivity pattern for all individual amino acids. The H{^{13}C}MQC spectrum with its $^1J_{CH}$ coupling

Table III: ^1H and ^{13}C (in Parentheses) NMR Assignments of Leucocin A in DMSO-d_6 containing 0.2% TFA[a]

residue	NH	CHα	CHβ	other
Lys 1	8.04	3.68 (51.6)	1.67 (30.3)	γCH$_2$ 1.27 (20.5); δCH$_2$ 1.50 (25.6); εCH$_2$ 2.70 (38.2); NH$_3^+$ 7.77
Tyr 2	8.50	4.48 (54.2)	2.90/2.63 (36.4)	3,5H 6.64 (114.8); 2,6H 7.02 (130.0)
Tyr 3	8.22	4.42 (53.8)	2.90/2.71 (36.4)	3,5H 6.62 (114.8); 2,6H 7.02 (130.0)
Gly 4	8.11	3.76 (41.6)		
Asn 5	8.15	4.58 (49.4)	2.52 (36.8)	NH$_2$ 7.42/6.95
Gly 6	8.23	3.82/3.70 (41.6)		
Val 7	7.77	4.21 (57.6)	1.88 (29.8)	γCH$_3$ 0.79 (18.7)
His 8	8.34	4.83 (50.4)	3.04/2.94 (27.0)	2H 8.88 (133.4); 4H 7.26 (116.9)
Cys 9	8.54	5.15 (*b*)	2.97/2.91 (41.0)	
Thr 10	8.45	4.39 (57.0)	4.52 (*b*)	γCH$_3$ 1.04 (18.9)
Lys 11	8.37	4.00 (54.5)	1.72/1.68 (29.8)	γCH$_2$ 1.40/1.47 (22.1); δCH$_2$ 1.54 (26.0); εCH$_2$ 2.75 (38.4); NH$_3^+$ 7.74
Ser 12	7.87	4.19 (56.4)	3.67/3.47 (60.6)	
Gly 13	7.58	4.25/3.55 (41.2)		
Cys 14	8.64	5.11 (*b*)	2.90 (43.6)	
Ser 15	8.14	4.48 (54.2)	3.59/3.62 (61.8)	
Val 16	8.04	4.28 (57.3)	1.87 (29.8)	γCH$_3$ 0.72 (18.6/17.6)
Asn 17	8.08	4.58 (49.4)	2.56/2.38 (36.8)	NH$_2$ 7.44/6.97
Trp 18	8.12	4.42 (53.8)	3.16/2.95 (26.9)	2H 7.14 (126.2); 4H 7.49 (118.1); 5H 6.93 (118.1); 6H 7.02 (120.7); 7H 7.29 (111.0); NH 10.71
Gly 19	8.23	3.68 (41.9)		
Glu 20	7.86	4.29 (51.4)	1.83/1.70 (27.1)	γCH$_2$ 2.20 (29.8)
Ala 21	7.98	4.22 (47.7)	1.16 (17.7)	
Phe 22	7.95	4.53 (53.4)	3.01/2.78 (36.8)	2,6H 7.21/7.14 (129/127.8/123.4)
Ser 23	8.02	4.30 (54.5)	3.62/3.54 (61.4)	
Ala 24	8.09	4.28 (48.3)	1.24 (17.7)	
Gly 25	8.06	3.73 (41.9)		
Val 26	7.76	4.09 (57.5)	1.88 (29.5)	γCH$_3$ 0.78 (17.8)
His 27	8.32	4.60 (51.0)	3.05/2.93 (26.6)	2H 8.93 (133.7); 4H 7.30 (116.9)
Arg 28	7.97	4.25 (52.1)	1.68/1.53 (28.7)	γCH$_2$ 1.47 (24.2); δCH$_2$ 3.09 (40.2); NH$_3^+$ 7.57
Leu 29	8.09	4.28 (50.6)	1.43 (40.2)	γCH 1.58 (23.8); δCH$_3$ 0.87 (22.7); εCH$_3$ 0.82 (21.2)
Ala 30	8.01	4.22 (47.8)	1.20 (17.6)	
Asn 31	8.06	4.51 (49.4)	2.53/2.45 (36.8)	NH$_2$ 7.38/6.93
Gly 32	8.03	3.72/3.67 (41.5)		
Gly 33	8.07	3.73/3.68 (41.5)		
Asn 34	8.06	4.51 (49.4)	2.53/2.45 (36.8)	NH$_2$ 7.38/6.93
Gly 35	8.04	3.60 (41.8)		
Phe 36	7.98	4.54 (53.4)	2.98/2.76 (37.3)	2,6H 7.21/7.14 (129/127.8/123.4)
Trp 37	8.21	4.46 (52.6)	3.18/3.06 (26.6)	2H 7.15 (126.2); 4H 7.51 (118.1); 5H 6.96 (118.1); 6H 7.06 (120.7); 7H 7.33 (111.0); NH 10.82

[a] 500-MHz (^1H) and 125-MHz (^{13}C) NMR spectra at 303 K in parts per million relative to Me_4Si (internal reference DMSO 2.49 ppm). See Supplementary Material for data in H_2O/D_2O/DMSO-d_6 (8:1:1, 0.2% TFA) at 283 K. [b] Not observed.

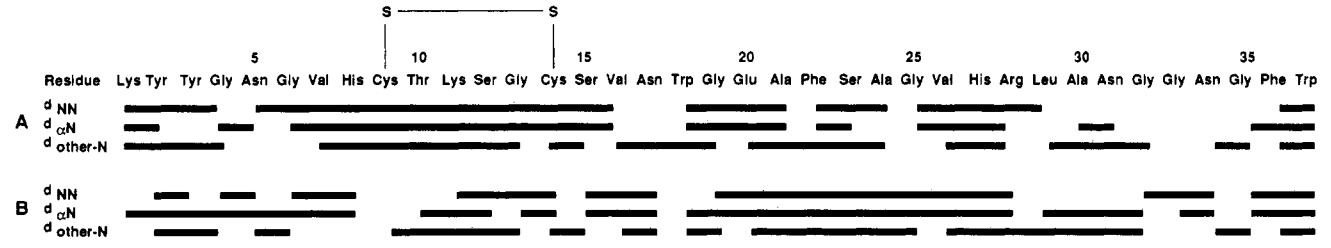

FIGURE 6: NOEs observed in leucocin A. The solid bars represent identified short-range NOEs: NH^i–NH^{i+1} (d_{NN}), C$_\alpha$H^i–NH^{i+1} ($d_{\alpha N}$), and CH^i(other)–NH^{i+1} ($d_{other-N}$) observed in (A) DMSO with 0.25% TFA at 303 K and (B) 90% water with 10% DMSO and 0.2% TFA at 283 K.

pattern connects these data with the already reconfirmed sequential proton chemical shifts. The H{^{13}C}MBC spectrum provides additional evidence for some of the interresidual connectivities via $^3J_{CH}$ couplings which in previous studies relied on NOESY data. However, it is difficult to identify all cross peaks in the H{^{13}C}MBC spectra without additional information since this experiment exhibits intensive spectral overlap and also does not distinguish between different $^nJ_{CH}$ couplings. Nevertheless, these spectra distinguish the proton and carbon resonances of both methionine methyl groups (Met 17 CH$_3$ at δ 2.10; Met 21 CH$_3$ at δ 2.08), which could not be definitively assigned in a number of the previous studies.

The backbone ^{15}N chemical shift assignments (Table II) are available from the H{^{15}N}MQC spectrum of [^{13}C,^{15}N]-nisin A (Figure 4). Dispersion of nitrogen resonances over about 35 ppm suggests rigid local structures within nisin A, in agreement with recent investigations which indicate such conformationally defined elements despite the high mobility and variation apparent for the whole molecule in aqueous solution (Van de Ven et al., 1991; Lian et al., 1991, 1992; Goodman et al., 1991). Comparison of one-dimensional ^1H NMR spectra obtained with and without heteronuclear (^{13}C or ^{15}N) decoupling affords reasonable estimates of the extent of isotopic enrichment in the nisin A samples (Figure 5). For example, upon application of ^{13}C decoupling, the amide hydrogens of residues 2, 5, and 33 (9.6–9.9 ppm) in [^{13}C,^{15}N]-nisin A appear as broad doublets due to $^1J_{N,H}$ couplings (ca. 90 Hz) together with singlets of species lacking ^{15}N in an

approximate integral ratio of 4:1. Using ^{15}N decoupling, the doublets collapse to the singlets which show splitting from long-range $^{n}J_{C,H}$ couplings. Since the *Anabaena* peptone used to produce this sample had 97% ^{13}C and 75 ± 5% ^{15}N, the results show that, as expected, there is no dilution of ^{15}N label during nisin production. In contrast, examination of the olefinic ^{1}H resonances of residues 2, 5, and 33 (5.4–6.63 ppm) and of both δ protons of histidine (residues 27 and 31) indicates different levels of dilution of ^{13}C label at various sites by unlabeled glucose added to the *L. lactis* fermentation. For example, the dehydroamino acid residues 2, 5, and 33 show 70–80% ^{13}C enrichments from the [^{13}C,^{15}N]peptone (97% ^{13}C) whereas the histidine carbons have >95% ^{13}C. The same effect is observed using the [^{13}C]peptone (50% ^{13}C) for which the corresponding isotopic enrichments are 30–35% and 50%, respectively. This is due to metabolic processes in *L. lactis* which appear to utilize some amino acids like histidine directly from the medium without de novo synthesis but mix the labeled (peptone) and unlabeled (glucose) carbon sources of other amino acids such as serine and threonine, which are the precursors to the dehydroamino acid residues. Such differential levels of labeling by the complex media could presumably be eliminated by replacement of unlabeled glucose with the highly enriched (>98% ^{13}C) U-^{13}C-labeled compound. The results clearly indicate that, unless very high levels of labeling are achieved, caution is necessary with quantitative aspects of NMR interpretation and in referring to peptides and proteins as "uniformly" labeled.

Labeling of Leucocin A and Its NMR Spectra. The nonlantibiotic bacteriocins from lactic acid bacteria possess considerable potential in food preservation (Klaenhammer, 1988; Stiles & Hastings, 1991), but because they often occur in small amounts and can be difficult to purify, a relatively small number of these peptides have had their primary sequence determined (Joeger & Klaenhammer, 1990; Rammelsberg et al., 1990; Hastings et al., 1991; Holo et al., 1991; Lyon et al., 1991; Mørtvendt et al., 1991; Muriana & Klaenhammer, 1991; Van Belkum et al., 1991a). One example studied in our laboratories, leucocin A from *L. gelidum* UAL187, inhibits a wide spectrum of lactic acid bacteria, meat spoilage bacteria, and food-related pathogens such as *Listeria monocytogenes* (Hastings & Stiles, 1991; Hastings et al., 1991). Isotopic labeling in conjunction with NMR spectral analysis is an ideal means of verifying the primary structure of such bacteriocins obtained from genetic sequencing and also provides a basis for investigation of three-dimensional solution structure. Since *L. gelidum* fails to produce the desired metabolite on defined media, a 1.7-L cultivation of this organism on the *Anabaena* peptone (30% ^{13}C) was used to generate 1.7 mg of [U-^{13}C]leucocin A. The NMR studies employed both this sample and unlabeled leucocin A; the latter was generated on a casamino acid medium as previously reported (Hastings et al., 1991). The tendency of leucocin A to aggregate in pure water and form gels could be suppressed by use of DMSO-d_6 (0.2% TFA) and 90% water/10% DMSO (0.2% TFA, unlabeled sample only). The assignment strategy was similar to that described above for nisin A with the ^{1}H spin systems in 2D DQF-COSY and 2D TOCSY spectra being linked with information in 2D NOESY data. Spectral acquisition at several temperatures (283 and 298 K for H_2O samples; 283, 303, and 313 K for DMSO samples) circumvents bleaching effect problems caused by solvent suppression. Transfer of information on interresidue NH–NH, C_αH–NH, and C_βH–NH connectivities from NOESY spectra back to the 2D TOCSY and DQF-COSY spectra was essential for completion of the ^{1}H NMR assignment (Table III) because of extensive resonance overlap resulting from the high number of glycine residues in this 37 amino acid peptide.

The ^{13}C assignments (Table III) for those carbons adjacent to protons were available via inverse H{^{13}C}MQC (Bax & Subramanian, 1986) experiments. All of the carbons could be identified in these spectra with the exception of C_α of both cysteines and the C_β resonance of threonine, which were not observable with a variety of acquisition parameters. The advantages of using [U-^{13}C]leucocin A from the *Anabaena* derived peptone are readily apparent. A 1 mM sample of the labeled leucocin gave excellent H{^{13}C}MQC spectra in about 6.5 h using 32 scans per increment, whereas a much more concentrated solution (4 mM) of the corresponding unlabeled material produced a comparable signal to noise ratio in 52 h employing 256 scans per increment. Clearly, the combination of labeling with *Anabaena* peptone and NMR analysis promises to be a useful tool for structure elucidation of bacteriocins from lactic acid bacteria in cases where limited amounts are available and the producing organisms require complex media for growth.

The NMR spectra of leucocin A display chemical shifts for amino acid residues near the disulfide bridge which are somewhat different from those determined for amino acids in a random coil peptides (Grathwohl & Wüthrich, 1974; Bundi et al., 1975). This suggests that conformations for those regions may be more defined, possibly with a β-sheet and a rigid turn starting from the disulfide bridge (residues 9–14). However, long-range interresidue NOEs typical for β-sheets or α-helices were not observed. The overall paucity of such NOEs under a variety of conditions (Figure 6) and preliminary calculations using FELIX and DSPACE software indicate that leucocin A does not possess clearly defined conformations in aqueous or DMSO environments. The lipophilicity of this molecule and its tendency to self-associate to form gels in water support the idea that, like other lactic acid bacteriocins, including nisin A (Gao et al., 1991), pediocin AcH (Bhunia et al., 1991), and lactococcin A (Van Belkum et al., 1991b), leucocin A acts at the membrane to release the cellular contents of sensitive bacteria. Studies on the mechanism of action and possible three-dimensional structure of leucocin A in model membranes are in progress.

ACKNOWLEDGMENT

We thank Dr. Christopher Lowe for helpful discussions and Dr. Thomas T. Nakashima for assistance in acquiring the NMR data.

SUPPLEMENTARY MATERIAL AVAILABLE

Table of ^{1}H NMR assignments of leucocin A in H_2O/D_2O/DMSO-d_6 (8:1:1, 0.2% TFA) (3 pages). Ordering information is given on any current masthead page.

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