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Structure Revision of the Lantibiotic 97518

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The lantibiotic 97518, produced by a *Planomonospora* sp., was reported as a 2194 Da polypeptide comprising 24 amino acid residues with five thioether bridges. It was assigned to the mersacidin subgroup of type B lantibiotics by Castiglione et al. (*Biochemistry* **2007**, *46*, 5884−5897) and named planosporicin. New analytical, chemical, and genetic data and reinterpretation of the published NMR chemical shifts enable structure revision of 97518. The resulting revision of the 97518 structure involves both a shift of two amino acids and a reorganization of two thioether bridges. With this revision, the lantibiotic 97518 becomes a clear member of the nisin subgroup of compounds.

Lantibiotics are ribosomally synthesized and post-translationally modified peptides produced by Gram-positive bacteria.[1] They contain the thioether-cross-linked amino acids lanthionines and/or methyllanthionines in addition to 2,3-didehydroalanines (Dha) and/or (*Z*)-2,3-didehydrobutyrines (Dhb). On the basis of their structural and functional features, the lantibiotics endowed with antibacterial activity are currently divided into two groups: type A are typically elongated, amphiphilic peptides, while type B are compact and globular.[2] Nisin is the prototype of type A lantibiotics, whereas actagardine and mersacidin belong to the type B subclass. Despite differences in shape and primary structure, both nisin- and mersacidin-type lantibiotics interact with the membrane-bound peptidoglycan precursor lipid II.[3] Lantibiotics have been isolated mostly from the order *Firmicutes*, while relatively few have been described from the *Actinomycetales*, with actagardine[4] and the recently described 107891[5] and 97518 (planosporicin)[6] as representative examples.

The lantibiotic 97518, produced by a *Planomonospora* sp., was reported as a 2194 Da polypeptide consisting of 24 amino acid residues with five thioether bridges. On the basis of the reported structure (**1**) and mode of action, 97518 was assigned to the type B lantibiotics. The lantibiotics 97518 and 107891, produced by the *Planomonospora*-related genus *Microbispora*, represent promising compounds to potentially treat infections caused by multiresistant Gram-positive pathogens. Inspection of the published structure (**1**) of 97518 highlighted similarity in amino acid sequence to nisin-type lantibiotics, including 107891, despite different thioether linkages. The first 12 residues of nisin fold back into the first two lanthionine rings, forming a cage-like structure that forms five intermolecular hydrogen bonds between the backbone amide functionalities of nisin and the pyrophosphate group.[7] The N-terminal two-ring system is maintained in a number of other lantibiotics, suggesting a common lipid II-interacting fold. In contrast, mersacin-type lantibiotics interact with lipid II through a different fold, proposed to be the highly conserved CTLTXEC propeptide sequence (X any amino acid).[8] The reported structure of 97518 (**1**) assigns thioether linkages between amino acids 3−11 and 7−18, thus resulting in neither the nisin- nor the mersacidin-type fold. However, the N-terminal 10-aa sequence of the linear precursor shares six identical amino acids with nisin, suggesting that thioether linkages different from those published would result in a nisin-type fold. Data are reported that result in the 97518 structure being revised as **2**, thus possessing a new amino acid sequence and two different thioether linkages from the structure **1** published by Castiglione et al.[6]

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Table 1. MS/MS ESI$^+$ of *m/z* 1097.7

m/z	fragment/ion
2076.9	1−23[−Ala(OH)SH]
1828.9	5−24(SH)
1573.7	7−24(SH)
1470.7	8−24
1387.6	9−24(SH)
1130.7	12−24
1097.7	$[M + 2H]^{2+}$
1088.6	$[M - 18 + 2H]^{2+}$
1039.1	$[M - Ala(OH)SH + 2H]^{2+}$
694.5	$[9-24(SH) - 18 + 2H]^{2+}$

Results and Discussion

Repetition of the NMR experiments confirmed the published data, but the determination of the lanthionine spin systems was difficult due to the overlapping signals belonging to 7-Ala and 11-Ala (structure **1**), which generated ambiguity on the thioether bridge positions and allowed more structures to be compatible with the experimental data. These spin systems were identified from the analysis of 2D $^{15}N-^{1}H$-HSQC-TOCSY data and from the analysis of the spectra at different temperatures. In particular, a diagnostic NOE association involving the Abu 8 suggested that the residue in position 9 is a proline. The sequential specific assignment indicates that the primary sequence obtained in the published data was not correct and that the Pro-Gly motif was in position 9−10 instead of position 23−24. Benzylamidation of the two carboxylic acids present in 97518 provided the dibenzylamide derivative **3**. The observed NOE signals of **3** highlighted that the carboxy-terminal amino acid could not be glycine, since the spin system of the carboxy-terminal benzylic NH consists of three peaks (one H_α and two H_β), suggesting instead an Ala at the C-terminus.

This anomaly was confirmed by MS/MS experiments. Among all the fragmentations observed (Table 1), three were particularly diagnostic to discriminate between **1** and **2**. The peak at *m/z* 2076.9 corresponds to the loss of a C-terminal Ala, thus confirming the NMR data. Furthermore, the peak at *m/z* 1130.7 matches fragment 12−24 of **2** and can only be explained by shifting the 23Pro-24Gly segment of **1** to positions 9 and 10 as in **2**. Finally, the peaks at *m/z* 1130.7 and 1470.7 are consistent with fragments containing intact rings, only compatible with a structure in which 20-Ala is connected with a residue having a position in the sequence not lower than 12. Thus, in the revised structure **2**, thioether bridges connect residues 3 with 7 and 13 with 20.

Confirmation of the revised structure **2** came from genome scanning of the producer strain (M. Sosio, unpublished data), which led to the sequence of the structural gene encoding the 97518 precursor, whose deduced amino acid sequence is ITSVSWCT-PGCTSEGGGSGCSHCC,[9] fully consistent with **2** and providing the location of Ser/Thr and Cys residues consistent with the thioether bridges as assigned in **2**.

In conclusion, the combination of MS spectrometry, NMR spectroscopy, chemical derivatization, and genomic techniques allowed revision of the 97518 structure involving both a shift of two amino acids and a reorganization of two thioether bridges. This result demonstrates that, in solving the structure of a lantibiotic, a combination of techniques should be employed to avoid the danger of misinterpretation when a single technique is applied. With this revision, the lantibiotic 97518 becomes a clear member of the nisin subgroup of compounds, strongly resembling the other lantibiotic 107891, also produced by a member of the *Streptosporangiaceae* family. This expanded family of Actinomycetales-derived antibacterial lantibiotics can provide valuable insights into structure−activity relationships of this promising class of antibiotics. A comparison of the 97518 and 107891 3-D structures will provide additional data on their relatedness and will be the subject of further research.

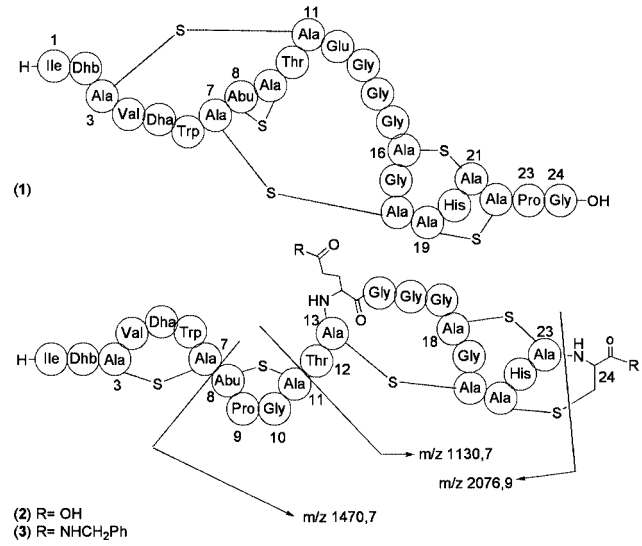

Figure 1. Previously published (**1**) and revised (**2**) chemical structures of the lantibiotic 97518 and its dibenzylamide derivative (**3**).

Experimental Section

General Experimental Procedures. NMR spectroscopy: 6.1 mg of 97518 was dissolved in a mixture of CD_3CN (60 μL)−H_2O(400 μL)−D_2O adjusted to pH 2 with DCl (20 μL). The dibenzylamide (5 mg) of 97518 (**3**) was dissolved in 100 μL of CD_3CN + 400 μL of H_2O (pH 2). Homonuclear ^1H and heteronuclear $^1H-^{13}C$ and $^1H-^{15}N$ NMR experiments were recorded at 600 MHz on a Bruker Avance spectrometer. The following experiments were performed: TOCSY with a mixing time of 20, 60, and 100 ms, NOESY with a mixing time of 300 and 700 ms, $^1H-^{15}N$ HSQC ($J = 90$ Hz), $^1H-^{15}N$ HSQC-TOCSY, $^1H-^{13}C$ HSQC ($J = 145$ Hz), and $^1H-^{13}C$ HMBC with $J^{^1H-^{13}C} = 8$ Hz for long-range correlation. The spectra were acquired at three different temperatures: 283, 298, and 313 K. The ES (Excitation Sculpting) sequence was used for water suppression. Spectra were analyzed with the program XEASY.[10] The complete sequence-specific ^1H resonance assignment was obtained following standard procedures.[11] MS spectrometry: The MS spectra were obtained with ESI in the positive mode by direct infusion using a Bruker Esquire 3000+ ion trap mass spectrometer. The doubly charged ion corresponding to 97518 shows an isotopic distribution (spacing 0.5 *m/z*) with *m/z* 1097.7 as lowest peak. MS/MS experiments on the doubly charged ion were performed with fragmentation amplitudes set at 0.7, 1.2, and 2 V. HPLC analysis: instrument, Shimadzu 2010; column, Merck Lichrosphere RP-18, 4 × 125 mm, 5 μm; flow rate, 1 mL/min; sample concentration, 1 mg/mL; detection, $\lambda = 230$ nm; inj. vol. = 20 μL; phase A: 0.1% aqueous TFA; phase B: MeCN. Linear gradient: time = 0 min, phase B = 10%; $T = 40$ min, phase B = 90%; followed by 10 min for reequilibration with phase B = 10%.

Preparation of Dibenzylamide of 97518 (3). To a stirred solution of 30 mg of 97518 (13 μmol, prepared as in ref 6) and 20 μL of $BnNH_2$ in 300 μL of DMF was added 14.2 mg of PyBOP (27 μmol), and the reaction mixture was kept under stirring at room temperature for 1 h. HPLC monitoring showed completion of the reaction after 2 h (retention time of **2** and **3** 15.5 and 19.5 min, respectively). The reaction was diluted with H_2O (0.5 mL) and the pH corrected to 3−4 by adding 20 μL of formic acid. The filtered solid was dissolved in 1 mL of a mixture of 0.1% TFA in 2:8 MeCN−H_2O and lyophilized. The final product was characterized with MS (2369.90 amu with $[M + 2H]^{2+}$ ion at *m/z* 1186) and with NMR.

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Supporting Information Available: The complete NMR assignment for 97518 and the NMR characterization of compound **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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