VIP Lantibiotics

DOI: 10.1002/anie.200905773

Labyrinthopeptins: A New Class of Carbacyclic Lantibiotics**

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Dedicated to Dr. Dieter Häbich

Lantibiotics are peptides that are ribosomally synthesized from bacteria such as staphylococci, lactobacilli, and actinomycetes. The common structural characteristic of lantibiotics is the noncanonical amino acid lanthionine (Lan, **1**; Figure 1), which confers conformational stability to the peptide.[1–4] The most prominent representative is nisin, which is a lipid II binder,[5] and has been known for its use as an antimicrobial food preservative for over 40 years.[6] The majority of studies on molecular targets and bioactivities are focused on potential applications of lantibiotics as anti-infectives.[7–10] Duramycin (Moli1901) is in phase II clinical trials for the treatment of cystic fibrosis because of its ability to increase chloride transport in airway epithelium.[11] Biosurfactant function in the life cycle of streptomycetes has been elucidated for some members such as SapB.[12]

Herein, we present the structures, the biosynthesis gene cluster, and the bioactivities of labyrinthopeptins, which are lantibiotics that contain labionin, an unprecedented carbacyclic, posttranslationally modified amino acid.

The culture extracts of the novel actinomycete *Actinomadura namibiensis* DSM 6313[13,14] attracted our attention because of their activity against the *Herpes simplex* virus. Active fractions of the extracts contained a peptide that was isolated by chromatographic methods. The high-resolution ESI-FTICR mass spectrum showed a mass of 984.3333 Da for the doubly charged sodium adduct of the compound, corresponding to a neutral monoisotopic mass of 1922.6872 Da and the molecular formula $C_{85}H_{110}N_{20}O_{24}S_4$ ($\Delta m/m = 0.7$ ppm). Amino acid analysis revealed Gly and the L-enantiomers of Ala, Thr, Leu, Asx, Cys, Phe, Glx, Trp (ratio 1:1:1:2:1:2:1:1:2). However, the total molecular mass of the detected amino acids indicated a considerable mass difference, which could not be correlated with known peptidic or lantibiotic posttranslational modifications. Resolution of the structure by ^{1}H NMR spectroscopy was impeded by broad signals in parts of the spectrum. The X-ray structure at 1.0 Å resolution (Figure 1) enabled interpretation of the analytical data and displayed several unique structural features. In view of its labyrinthine structure, the compound was named labyrinthopeptin A2 (**2**).

Labyrinthopeptin A2 has a globular structure that consists primarily of hydrophobic amino acids. Formally, the structure can be dissected into two nonapeptides. Each peptide bears a C-terminal Cys residue that forms a disulfide bond, which is a comparatively rare modification in lantibiotics, but is found for sublancin 168 from *B. subtilis*.[15] Each nonapeptide contains a tetrapeptide (ring A) and a pentapeptide (ring B) that share a quaternary αC atom; labyrinthopeptin A rings are formed by a methylene group between the αC atoms of Lab1/Lab10 and Lab4/Lab13 (Figure 1). A carbacyclic side-chain linkage is unprecedented in peptides and proteins. We propose the name labionin (Lab) for the corresponding amino acid (Figure 1). Labionin **3** represents an αC quaternary substituted amino acid with a subtle structural resemblance to α-aminoisobutyric acid (Aib) or isovaline (Iva), which are incorporated in fungal peptaibol-type antibiotics.[16] The stereocenters of **3** can be assigned to (2*S*,4*S*,8*R*)-labionin (Lab), which is consistent with the configuration of (2*S*,6*R*)-lanthionine of other lantibiotics.[1,2] The formation of the 11-membered ring that involves **3** forces the peptide backbone into a conformation with *cis*-amide bonds between Asp2–Trp3 and Thr11–Gly12, respectively (Figure 1). The presence of *cis*-amide bonds and the absence of a hydrogen bond between Lab1–Lab4 and Lab10–Lab13, respectively, show that the turn motif in **2** is clearly different from a β-turn motif.

Subsequent identification of the biosynthetic gene cluster was performed from a cosmid library of *A. namibiensis* by means of degenerated primer probes, followed by sequencing

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[**] This research was supported by grants from the Deutsche Forschungsgemeinschaft (DFG SU239/8-1) and the German Ministry of Education and Research BMBF (no. 0315198). We thank Graeme Nicholson for ESI-FTICR-MS and amino acid analysis measurements, Ulrike Jannsen and Claus Lattemann for their work on resupplying the compounds, and Ronja Ring for support in the behavioral study.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200905773.

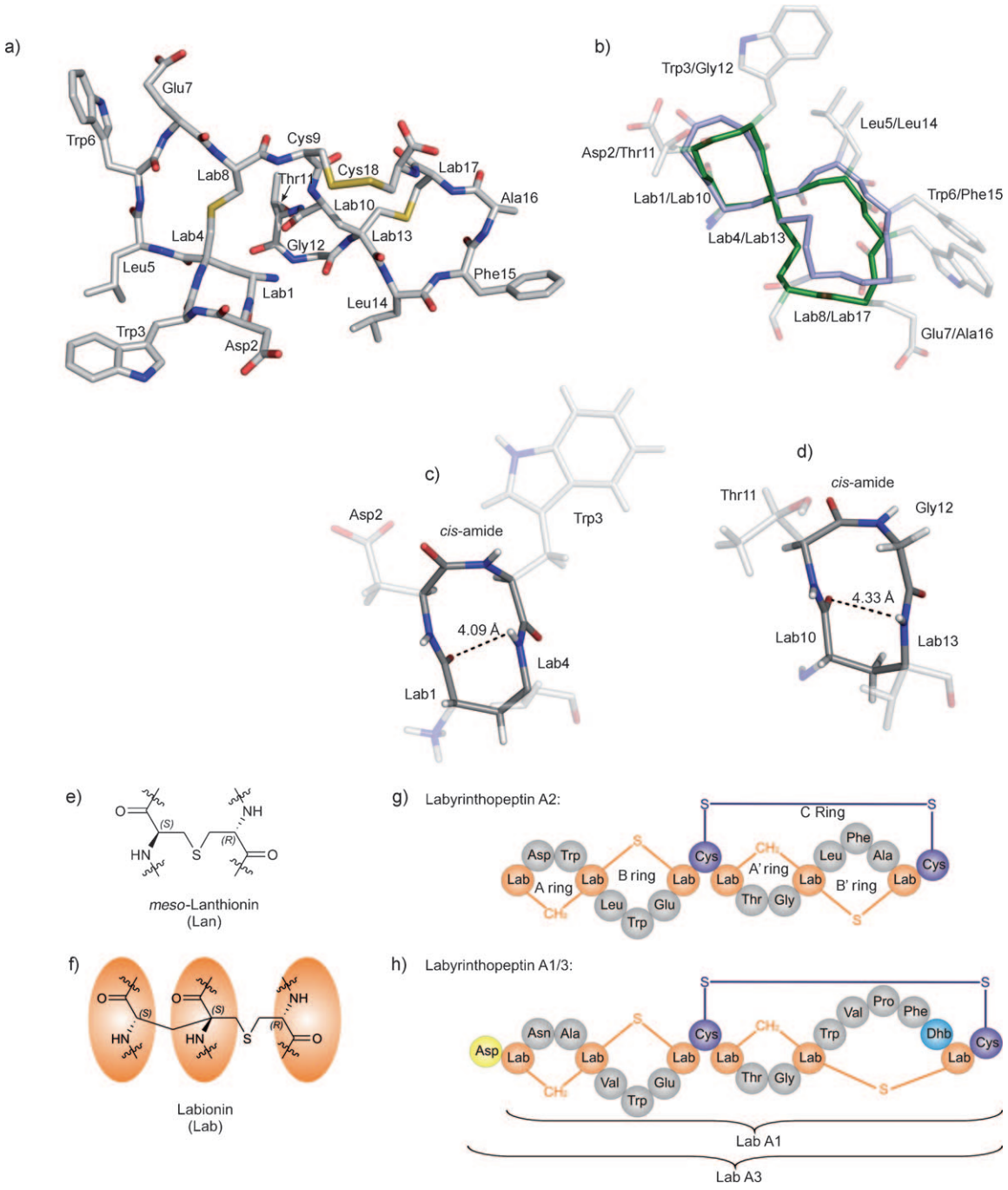

Figure 1. Structural data. a) Crystal structure of **2**. Stick model of the 3D molecular structure; gray C, blue N, red O, yellow S. b) Overlay of the A–B (green) and A′–B′ (purple) moieties after fitting the central αC atom and the four atoms bonded to it. The two ring systems display a high conformational similarity. c, d) Representation of the *cis*-amide turns of the A-ring (Lab1-Asp2-Trp3-Lab4) and of the A′-ring (Lab10-Thr11-Gly12-Lab13) formed by the methylene bridge of labionin. e, f) Amino acids lanthionine (Lan) **1** and labionin (Lab) **3** incorporated in lantibiotic structures. g, h), schematic primary structures of **2**, **4**, and **5**. Labionin A/A′ rings are formed by a methylene group between αC atoms of Lab1/Lab10 and Lab4/Lab13. B/B′ rings are formed by a thioether bridge. **4** is a degradation product of **5** formed by cleavage of the N-terminal Asp. Ala = alanine, Thr = threonine, Leu = leucine, Asx = asparagine/aspartic acid, Cys = cysteine, Dhb = didehydrobuyrine, Glx = glutamine/glutamic acid, Phe = phenylalanine, Ser = serine, Thr = threonine, Trp = tryptophan.

of the cosmid (see Supplementary Information). A 6.4 kb DNA sequence encodes five genes assigned to the labyrinthopeptin biosynthesis gene cluster (*lab*; Figure 2). The DNA sequence of the structural gene *labA2* (38 amino acids) of **2** has a 20 amino acid leader peptide and reveals a Ser-Xxx-Xxx-Ser-Xxx-Xxx-Xxx-Cys motif as precursor amino acids for **3** (Figure 2). Directly upstream of *labA2*, the gene *labA1/A3* codes for the structural gene of labyrinthopeptins A1 and A3 (leader peptide 20 and 19 amino acids, structural gene 20 and 21 amino acids, respectively). Genes *labT1* and *labT2*

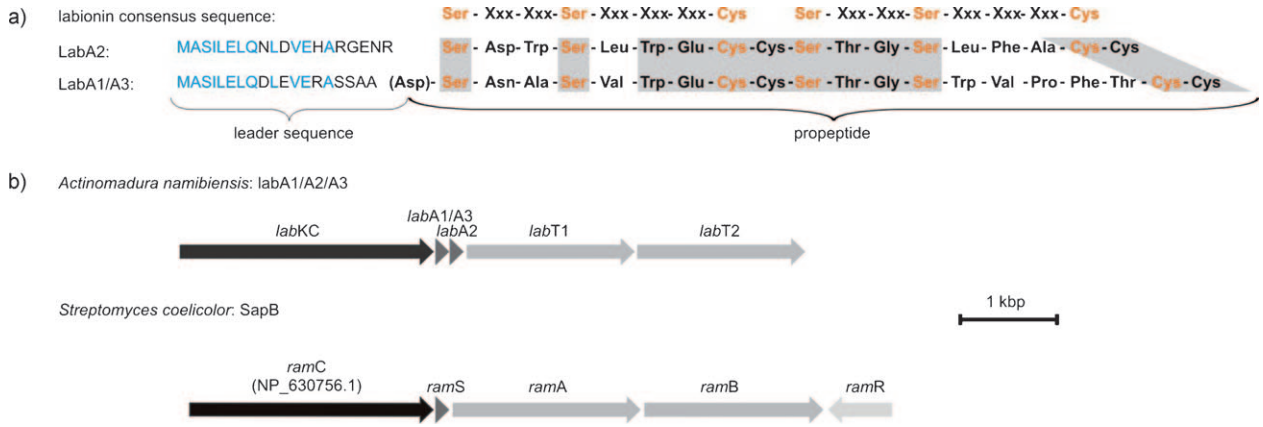

Figure 2. Biosynthesis of labyrinthopeptins. a) Peptide sequences of the prepropeptides of **2**, **4**, and **5** as transcribed from *labA1* and *labA2*. Identical residues of the leader sequences (one-letter code) are depicted in blue, and identical residues of the propeptides are shaded in gray. Amino acids Ser, Ser, and Cys are the biosynthetic precursors for labionin. b) Annotation of genes *labKC* (Ser/Thr kinase cyclase), *labA1/A3* (structural protein of **4** and **5**), *labA2* (structural protein of **2**), *labT1* and *labT2* (ABC transporter), identified in the labyrinthopeptin gene cluster. Comparison with the gene cluster of *Streptomyces coelicolor* encoding for SapB.

code for ATP-dependent ABC transporters[17] with a putative export function for labyrinthopeptins. The gene *labKC* codes for a bifunctional protein with an N-terminal Ser/Thr kinase function and a C-terminal lanthionine cyclase function. A thiol–disulfide oxidoreductase involved in disulfide formation (C ring), which was found essential for sublancin 168,[15] as well as a protease for cleavage of the prepeptide were not identified within the borders of the cosmid.

Two other compounds, labyrinthopeptins A1 (**4**) and A3 (**5**), were found in culture filtrates of *A. namibiensis* (see the Supporting Information). At longer fermentation times, **5** was converted into **4**. In fermentation extracts, the amount of **2** was approximately equal to that of **4** and **5**. This observation is consistent with a common transcription of *labA2* and *labA1/A3* from the same gene cluster, and implies similar processing efficiencies of the prepropeptides by LabKC and proteolytic enzymes. A combination of the genetic information, the structural data for **2**, and the data from mass spectrometric and amino acid analysis enabled the structural assignment of **4** and **5**, respectively (Figure 1). The B′ ring of **2** is extended to a heptapeptide with didehydrobutyrine (Dhb) derived from Thr, and **5** possesses an additional N-terminal Asp compared to **4**.

In vitro compound profiling confirmed the antiviral activity found in the original extracts (see the Supporting Information). In further experiments, **2** was tested in vivo in a spared nerve injury mouse model of neuropathic pain.[18] Intravenous administration of **2** (0.01–3.0 mg kg^{-1}) led to a significant attenuation of tactile allodynia with an ED_{50} value of 50 μg kg^{-1}. The efficacy reached 100% and was stable over the observation period of 6 h. 24 h after application, the antiallodynic effect had disappeared, as seen for the comparator gabapentin at 3 mg kg^{-1} (Figure 3 and the Supporting Information).

Taken together, our observations suggest that the labyrinthopeptins represent a new class of carbacyclic lantibiotics that can be assigned to type III lantibiotics according to the gene cluster.[2] In our current model, labyrinthopeptins are synthesized as prepropeptides and posttranslationally processed by the didomain protein kinase-cyclase LabKC, which subsequently catalyzes reactions such as serine phosphorylations, dehydrations of phosphoserines to didehydroalanines, and cyclizations. Precedence for an ATP-dependent phosphorylation step has been reported for LctM in lacticin 481 biosynthesis.[19,20] Lanthionine formation in other lantibiotics occurs through a Michael-type addition, followed by protonation of the enolate. We assume that in the case of labyrinthopeptin biosynthesis, the enolate intermediate is not quenched by protonation, but undergoes a second Michael addition in situ with a second didehydroalanine. Final biosynthetic steps comprise the N-terminal processing of the prepeptide by proteases, disulfide bond formation, and export into the extracellular space.[1,2]

The arrangement of genes and the leader sequences of the structural genes *labA1/A3* and *labA2* display significant homologies to gene clusters present in other actinomycetes (Figure 2). This observation particularly applies for the *ram* gene cluster of the model organism *Streptomyces coelicolor* with SapB as the biosynthetic product.[12] The common sequential alignment of a Ser/Thr kinase (*ramC*), a structural protein (*ramS*) and ABC transporters (*ramA, ramB*) underlines the evolutionary relationship of both gene clusters. The structural model of SapB suggests the existence of lanthionine/Dha pairs rather than labionin.[12]

Except for the transcriptional regulator sequence *ramR*, the *ram* gene cluster displays no additional genes, for example, for proteolytic processing of the prepeptide. The presence of *lab* homologous gene clusters in *Streptomyces avermitilis*, *Streptomyces griseus*, and the erythromycin producer *Saccharopolyspora erythrea* with yet unknown biosynthetic products suggests a significant role for this lantibiotic type in actinomycetes (see the Supporting Information).

Profiling experiments aimed at finding bioactivities of labyrinthopeptins led, in addition to moderate antiviral effects, to neuropathic pain[21] as an indication. Antiallodynic effects in vivo have not been described to date for lantibiotics,

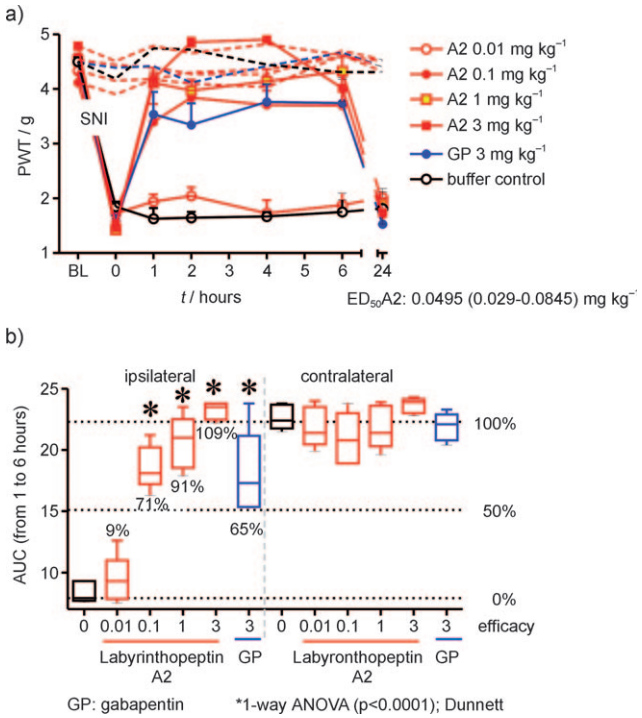

Figure 3. Spared nerve injury (SNI) model of neuropathic pain. a) The tactile allodynia was estimated before and seven days after surgery (baseline (BL) = before and 0 h = seven days after injury). After intravenous application of **2**, gabapentin or vehicle (ethanol/solutol/phosphate buffered saline 1:1:18), the tactile allodynia was assessed by paw withdrawal threshold (PWT) measurements over 6 h and after 24 h. Data are given as mean values with SEM (N = 5/group). The dotted lines represent contralateral measurements. b) Area under the curve (AUC) calculations from 1 to 6 h after intravenous application. The results are separated for the neuropathic hind limb (ipsilateral) and for the non-affected healthy hind limb (contralateral). The median of the ipsilateral vehicle group was set to 0% efficacy and the median of all contralateral hind limbs as 100% efficacy. The boxes represent the median and the quartiles as well as the extreme values of the group. Statistical differences as indicated.

but are well-known for natural peptides from spider toxins[22,23] or cone snails.[24] The most prominent example is ziconotide, a conotoxin approved for the treatment of severe chronic pain.[25] Given their novel chemical architecture and the encouraging in vivo results, the labyrinthopeptins can be considered as an attractive novel lead series with potential applications in treatment of neuropathic pain. These compounds extend the structural space of lantibiotics by an unprecedented carbacyclic modification, yielding the new amino acid labionin. The enzymatic, posttranslational modification represents a new route to C–C bond formation in nature. The mechanistic details of this reaction will be the subject of future studies.

Experimental Section

CCDC 721326 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif and the DNA sequence has been deposited in the EMBL Nucleotide Sequence Database under accession no.: FN178622.

Received: October 14, 2009
Published online: January 15, 2010

Keywords: antibiotics · antipain activity · lanthionine · lantibiotics · peptides

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