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Tying the Knot: Making of Lasso Peptides

Gabriele Bierbaum[1] and Andrea Jansen[1,*]
[1] Institute for Medical Microbiology, Immunology and Parasitology, Sigmund-Freud-Strasse 25, 53105 Bonn, Germany
*Correspondence: andrea.jansen@mibi03.meb.uni-bonn.de
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In this issue of *Chemistry & Biology*, Duquesne and colleagues [1] describe the biosynthetic machinery of microcin J25, an antibacterial peptide that adopts an exceptional three-dimensional structure resembling a knot. The dedicated enzymes, McjB and McjC, were identified and employed to produce this modification in vitro.

The problem of increasing antibacterial resistance, that modern medicine faces today, has resulted in quite a number of studies that deal with natural antibacterial products and their biosynthesis. Microcins are defined as small antibacterial peptides that are produced by members of the Enterobacteriaceae. The designation "microcin" was coined to set off the low molecular weight antibacterial peptides (<10 kDa) against the large protein bacteriocins (e.g., the colicins) that are also produced by these bacteria. All microcins are ribosomally synthesized and they are often posttranslationally modified. The microcin group comprises peptides that differ widely with respect to their structure and mode of action. For example, microcin B17, a peptide that contains thiazole and oxazole rings, targets the DNA gyrase, microcin C7, which carries a C-terminal adenosine monophosphate, inhibits protein biosynthesis [2], and microcin E492 permeabilizes the cytoplasmic membrane in its unmodified as well as in its modified form [3].

Microcin J25 is characterized by a very unusual and fascinating structure: a so-called "lasso peptide" [4]. Lasso peptides are short peptides (16–21 amino acids) that contain an N-terminal ring structure that is closed by an amide bond between the N-terminal amino acid and the side chain carboxyl group of a glutamic or aspartic amino acid residue in position 8 or 9. The C-terminal tail is threaded through this ring structure and forms a loop or noose, which, in the case of microcin J25, is stabilized by bulky aromatic residues in the C terminus (Phe19 and Tyr20) that anchor the loop above and below the ring (Figure 1). The resulting backbone structure resembles a lasso and is very stable, thus, even after cleavage of the loop by proteases, the C-terminal part will stay anchored in the ring [5].

Several other examples of lasso peptides have been described so far. Although their modes of action are very diverse, the majority of these peptides bind to proteins. For example, the group includes MS-271, an inhibitor of the calmodulin-activated myosin light chain kinase [6], anantin, a peptide binding to the atrial natriuretic factor (ANF) [7], and two closely related peptides, NP-06 and RP 71955, which are active against human immunodeficiency virus 1 [8, 9]. The lariatins A and B [10] and microcin J25 are the only lasso peptides with antibacterial activity. Microcin J25 is produced by and is active against *Escherichia coli* strains. Furthermore, it inhibits growth of some *Salmonella* serovars. It passes the outer membrane, taking advantage of the FhuA iron siderophore transporter [11], and is taken up into the cytoplasm via other membrane proteins (Smb, TonB, ExbD and ExbB). Intracellularly it acts as an inhibitor of the RNA polymerase and stimulates the production of superoxide [12].

The main producers of lasso peptides characterized so far are members of the actinobacteria and belong to the genera *Streptomyces*, *Microbiospora*, and *Rhodococcus*. In order to synthesize a lasso peptide, like microcin J25, the prepeptide has to be folded in the correct manner, with the N terminus embracing the C terminus. Subsequently, the leader peptide cleavage and the ring formation need to take place. The enzymes that catalyze these maturation steps were unknown until now. For the first time, Duquesne and coworkers [1]

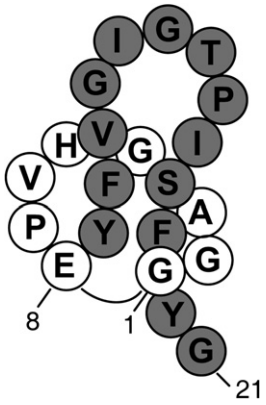

Figure 1. The Structure of Microcin J25
The N-terminal amide ring is shown in white and the C-terminal loop is shown in gray.

demonstrate the activity of the maturation enzymes in vivo and in vitro, which marks a major progress in our comprehension of the biosynthesis of lasso peptides.

McjB and McjC are encoded in the open reading frames adjacent to the structural gene of McjA, but they are transcribed divergently. A fourth protein, McjD, is a dedicated transport protein, also involved in producer self- protection [13]. First, the authors performed knockout and complementation experiments with McjB and McjD, which demonstrated that both enzymes are essential for the maturation of microcin J25. Next, both enzymes were cloned, expressed as His-tag proteins, and purified. The authors also cloned the His-tag construct of the precursor peptide of microcin J25, a prepeptide consisting of 58 amino acids. This prepeptide, with a 37 amino acid leader sequence, does not display antibacterial activity and seems to be quickly degraded by proteases. In vitro both enzymes, as well as ATP, $MgCl_2$, and DTT, were required for the maturation reaction to proceed and yield the active peptide, indicating that the combination of McjB and McjC is sufficient for the complete posttranslational modification. The presence of the leader sequence was essential for the modification. Finally, the group tested the influence of protease inhibitors on the reaction, since the cleavage of the leader had to precede ring formation, and demonstrated that inhibitors of serine proteases arrested the modification reactions as expected.

Many of the data shown here are reminiscent of results that were obtained with small Gram-positive bacteriocins, the lantibiotics. The mature forms of these peptides contain intramolecular thioether ring structures that are introduced by dedicated enzymes [14]. Lantibiotics also possess ribosomally made prepeptides that comprise N-terminal leader sequences and are considerably longer than the mature peptides. The leader peptides are needed for recognition by the modification enzymes and the unmodified or partially modified prepeptides are not antibacterially active and often susceptible to proteolytic degradation.

Since it had not been possible to isolate and analyze the intermediate product, in silico analyses were performed to obtain information on the substrate specificities of the two enzymes. McjC shows similarity to β-lactam synthetases and class B asparagine synthetases. Both enzyme classes catalyze the formation of the amide bonds and therefore McjC may be involved in formation of the N-terminal ring. McjB bears resemblance to transglutaminases and cysteine proteases and might act as the leader peptidase. However, the in vitro reaction was unable to proceed when serine protease inhibitors were added. This might indicate that either McjB is a serine, and not a cysteine, protease, or McjA, which shows some similarity to serine proteases, acts autocatalytically. This issue is yet to be resolved and warrants further work.

Surprisingly, similar gene clusters encoding putative lasso peptides were found in several genomes. They are present in the γ-proteobacteria *Burkholderia thailandiensis*, *B. pseudomallei*, and *B. mallei*, as well as in the α-proteobacteria *Caulobacter* sp. K31 and the marine bacterium *Sphingopyxis alaskensis*. Although not all of these gene clusters contain the homologous export protein, their presence clearly indicates that lasso peptides are far more widespread than formerly thought.

The successful in vitro reconstitution of the lasso peptide posttranslational modification system now opens the possibility for testing these enzymes in the in vitro peptide engineering assays. Therefore, this paper represents a stepping-stone that might enable biotechnology to "tie the knot" in peptides and design novel peptides with an enhanced stability, defined conformations, and yet unforeseen properties.

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