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Entanglement

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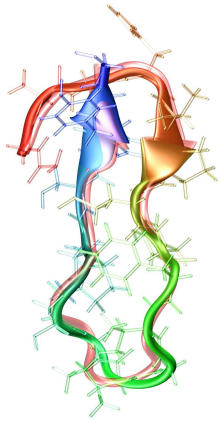
Getting tangled into knots is rarely a desirable situation. Yet there is a protein whose entanglement is not only profitable but also so final that it can kill off bacteria that interfere with its host's feeding or living space. Microcin J25 is a small antibacterial peptide synthesized by certain strains of *Escherichia coli* during times of hardship. Its knotted structure is such that it interferes with the victim's RNA polymerases hindering RNA polymerization and hence protein synthesis. As a consequence, the targeted bacteria die off leaving refreshment and room for their rivals.

The 3D structure of microcin J25 has been in the midst of heated research since the late 1990s. First it was thought that the 21 amino-acid peptide was cyclic, and that the structure folded over onto itself creating a compact globular protein. This was perfectly conceivable since a number of cyclic proteins had already been discovered – such as kalata B1[1] for instance. However, a number of scientists remained skeptic. Thanks to three cystine bridges and a particular twist in its structure, kalata B1 is extremely stable and resistant to degradation. Microcin J25, however, appeared to have no cystine bridges at all and nothing but a hypothetical backbone link between the N- and the C-termini. Such a structure could not account for its high stability and near impossibility to denature. What is more, synthetic cyclic microcin proved to be inactive. The structure needed to be rethought.

In 2003, a number of research groups simultaneously came up with another structure: that of a lassoed tail or a lariat protoknot… A structure never encountered before and which agreed with the spectral characteristics of the native peptide – something that the cyclic peptide had never shown. The general aspect of the 3D structure of the protein remained the same, i.e. small and globular, though it was not cyclic anymore but resembled more a lasso whose tail folds over and enters the noose. The noose itself is created by the N-terminal first eight amino acids, which fold into a circle to form a backbone/side-chain amide linkage. The tail – which consists of amino acids 9 to 21 – bends over and enters the noose by its C-terminal tip, with amino acids 19 and 20 straddling on either side.



Microcin J25

Courtesy of Fabrice David, SIB Geneva

The structure is hugely stable. The tail does not slip further through the noose but is held firmly in place by way of the straddling residues. This led researchers to suggest that such an irreversible entanglement cannot involve the formation of a ring into which a tail is simply slipped. Rather it would involve some kind of chaperone which pre-folds the tail into place, and around which the noose is tightened in a

[1] Protein Spotlight issue 20

subsequent step. The steps which lead to microcin's final lasso conformation are not known but its gene is accompanied by two others on the same plasmid, and their products could well be part of the microcin biosynthetic pathway.

How does microcin hinder RNA polymerization in other bacteria? RNA polymerase is a complex multisubunit molecule that is shaped like a crab's claw, the cleft of which runs along DNA to transcribe the messenger that will be translated into a protein sequence. RNA elongation demands a source of nucleoside phosphates – RNA polymerization substrates – which are thought to stream through a small channel into the cleft where RNA elongation is performed. Currently, it is thought that microcin floats into this small channel and just clogs it up so that all RNA substrate traffic is brought to a standstill. As a result, the nucloside phosphates cannot get past to reach the polymerase's catalytic site where they are added to a growing RNA molecule.

The discovery is exciting because until now ribosomes – rather than RNA polymerases – have been targets for designing antibacterial drugs. Getting to know proteins which inhibit the proper function of RNA polymerases is informative on two fronts: how RNA polymerases work and how their inhibitors work. To date, there is one well-known inhibitor of bacterial RNA and that is Rifampicin, which binds to the RNA molecule in progress stunting its elongation. Rifampicin and its derivatives are widely used to treat mycobacterial infections such as tuberculosis. A greater understanding of microcin's 3D structure coupled to that of the RNA polymerase's secondary channel could well provide viable targets for designing antibacterial drugs in a near future. Microcin is an inhibitor of Gram-negative bacteria; however, if its binding site is known in detail, tinkering with it could provide microcin variants that would be able to clog RNA polymerase channels of Gram-positive bacteria and even eukaryotes. All thanks to a knot.

Cross-references to Swiss-Prot

Microcin J25, *Escherichia coli*: Q9X2V7

References

1. Wilson K.-A., Kalkum M., Ottesen J., Yuzenkova J., Chait B.T., Landick R., Muir T., Severinov K., Darst S.A.
   Structure of microcin J25, a peptide inhibitor of bacterial RNA polymerase, is a lassoed tail
   J. Am. Chem. Soc. 125:12475-12483(2003)
   PMID: 14531691

2. Bayro M.J., Mukhopadhyay J., Swapna G.V.T., Huang J.Y., Ma L.-C., Sineva E., Dawson P.E., Montelione G.T., Ebright R.H.
   Structure of antibacterial peptide microcin J25: a 21-residue lariat protoknot
   J. Am. Chem. Soc. 125:12382-12383(2003)
   PMID: 14531661

3. Rosengren K.J., Clark R.J., Daly N.L., Göransson U., Jones A., Craik D.J.
   Microcin J25 has a threaded sidechain-to-backbone ring structure and not a head-to-tail cyclized backbone
   J. Am. Chem. Soc. 125:12464-12474(2003)
   PMID: 14531690

4. Adelman K., Yuzenkova J., La Porta A., Zenkin N., Lee J., Lis J.T., Borukhov S., Wang M.D., Severinov K.
   Molecular mechanism of transcription inhibition by peptide antibiotic microcin J25
   Mol. Cell 14:753-762(2004)
   PMID: 15200953

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