Membrane-binding and cellular uptake of cyclotides: scaffolds that stabilize and deliver drugs

S.T. Henriques, Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia.

Cyclotides, cyclic disulfide-rich peptides from plants, are ultra-stable molecules that have inspired applications in drug design as they can be used as scaffolds to stabilize linear bioactive sequences able to inhibit protein-protein interactions. Recently, they have also been shown to possess cell-penetrating properties. The combination of their remarkable stability and cell-penetrating properties opens new avenues for the application of cyclotides as a stable delivery system able to cross cell membranes and inhibit intracellular proteins involved in cancer pathways. To realize and optimize the application of cyclotides as a drug framework and delivery system, we studied their ability to enter mammalian cells. In particular we have studied the internalization of two cyclotides: kalata B1, a cyclotide belonging to Mobius subfamily; and MCoTI-II, a cyclotide that belongs to the trypsin inhibitor family. We have designed and synthesized a series of kalata B1 and MCoTI-II analogues and conducted structure-activity relationship studies using surface plasmon resonance, nuclear magnetic resonance spectroscopy, mass spectrometry, confocal microscopy and flow cytometry. We have shown that kalata B1, a globally-neutral, membrane-active peptide, enters cells *via* both endocytosis and by direct membrane translocation. Both pathways are initiated by targeting phosphatidylethanolamine phospholipids at the cell surface. MCoTI-II is a positively-charged peptide and unable to bind to cell membranes that enters cells *via* endocytic pathways. Based on structure-activity relationship studies we have re-designed both MCoTI-II and kalata B1 to improve their internalization properties and ability to target cancer cells. Our mode-of-action studies and design efforts to improve cellular uptake show that cyclotides can be reengineered to stabilize a linear peptide and to optimize their internalization properties and highlight the potential of these peptides as drug leads for the modulation of traditionally ‘undruggable’ targets, such as intracellular protein-protein interactions involved in cancer pathways.