Modulating the helicity of cell penetrating peptides to optimize activity *in vitro* and *vivo*

Abstract

New therapies with biomacromolecules are often restricted to extracellular targets due to insufficient cellular uptake and low bioavailability. One approach to solve the delivery issue is the use of cell penetrating peptides (CPPs), which can transport hydrophilic cargo across hydrophobic membrane barriers without causing cellular leakage. Our experiments aim at the elucidation of the membrane binding properties and conformational features of four different amphiphlic CPPs from Hoffmann-La Roche, to be compared in the linear form with their stapled analogues. In each peptide, a lactam-staple was inserted in a suitable position to induce a helical conformation, to enhance the helicity in the absence of a lipid membrane and thereby improve translocation activity. Using circular dichroism (CD), we determined the degree of helicity of the linear and stapled peptides, both in aqueous buffer and when bound to lipid vesicles. In macroscopically alined membrane samples, Oriented CD (OCD) then revealed the alignment of the molecules relative to the bilayer surface. In order to compare the effect of lipid composition and membrane curvature on the lipid-induced helix content of the peptides, we employed specific mixtures to mimic different kinds of cell membranes.

To assess the biological activity of the different CPPs, their *in vivo* cell uptake was measured via carboxyfluorescein-labelled analogues in different cell lines (Cooperation with RPF-ID: 272). Additionally, the uptake efficiency and leakage behaviour were examined using a fluorescent *in vitro* assay based on lipid vesicles. To that aim, additional coumarin-labelled peptide analogues had to be synthesized and purified. Our aim is to find a correlation between the biological uptake efficiency in eukaryotic cells and the different effects of membrane permeation/leakage on artificial lipid vesicles. Most interesting will be the relationship between uptake efficiency and peptide structure, lipid composition, membrane curvature, and aggregation tendency of the CPPs, as will be presented in this study.