Peptide Inhibitors of Bacterial Topoisomerases: Studies of Delivery into Bacterial Cell by Cell Penetrating Peptides.

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The *ccd* toxin-antitoxin (TA) system of the F plasmid encodes CcdB, a 11.7 kDa protein that poisons *Escherichia coli* DNA gyrase, unique type IIA topoisomerase able to introduce supercoils into DNA. CcdB forms a stable complex with the catalytic subunit of the DNA gyrase that is important for replication and transcription in that it relieves supercoiling in DNA by creating a transient break in the double helix. Recently, based on CcdB structure, we produce *CcdBET2*, a peptide analogue with an inhibition of supercoiling activity very close of obtained for CcdB (IC_{100} = 10 µM). Unlike of CcdB, *CcdBET2* inhibited the relaxation reaction of Topoisomerase IV (Topo IV), a second type II topoisomerase, with IC_{100} of 5 µM. Despite inhibitory activity *in vitro*, *CcdBET2* in solution was unable to inhibit the bacterial growth, due the poor permeability of the bacterial membrane. Bacterial membranes are generally impermeable to large hydrophilic molecules, and the limits the use of many useful compounds, as *CcdBET2.* To overcome this limitation we tested several cell penetrating peptides (CPP) to carry the CcdB analogues into cells. Selected CPP were fused with N-terminus of CcdB analogues and were tested about inhibition of DNA gyrase and Topo IV activity by agarose gel electrophoresis and by growth of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa*. The fused of CPP did not change the inhibition of topoisomerase activity and was able to inhibit *Bacillus subtilis* with a MIC of 50 µM. Our results showed that CPP are able to delivery CcdB analogues into bacterial cells.

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