New Peptide Inhibitors of Bacterial Topoisomerases

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The ParE-ParD is a toxin-antitoxin system encoded on a broad host range. ParE, the toxin, is a protein of 12 KDa encoded by gene parE. ParD, a small protein of 9 KDa encoded by gene parD is the antitoxin able to neutralize the ParE action. ParE inhibits DNA gyrase activity and thereby blocks DNA replication. Recent studies, employing peptides from E.coli ParE, showed that the toxic activity of the ParE also occurs by inhibition of Topoisomerase IV activity. Although found in a wide variety of microorganisms, the cellular function of ParE is not fully elucidated. Thus, its cytotoxic mechanism remains to be determined. As an approach for understanding of this mechanism we have designed and synthesized new linear analogues of ParE and investigated the ability of peptides to inhibit DNA topoisomerases activities. Based on previous structural data of E. coli ParE, new peptides were designed and synthesized by SPPS. Gel electrophoresis assay was employed to evaluate the ability of the peptides to inhibit the supercoiling reaction of gyrase and relaxation reaction of topoisomerase IV. A 4.7 KDa peptide (named ParERM3) showed complete inhibition of DNA gyrase activity, with an IC$_{100}$ of 20 μmol.L$^{-1}$. Differently of wild type ParE, this peptide analogue was able to inhibit the topoisomerase IV activity with lower IC$_{100}$ value (10 μmol.L$^{-1}$). The insertion of the LNIES sequence at the C-terminal extreme of ParERM3, rendered a peptide with less toxicity (IC$_{100}$ of 35 and 50 μmol.L$^{-1}$ for Gyrase and Topoisomerase IV, respectively). The difference between IC$_{100}$ values indicates a decrease in the binding with the enzymes. The random conformation of LNIES probably allows a close proximity of its amino acid side chains to the two highly conserved amino acids (H88 and M91) preventing the molecular interactions responsible for enzymes inhibition. Our results suggest the use of ParERM3 peptide as a promising antimicrobial agent.

Key Words: peptides, topoisomerases, toxin, antitoxin, ParE, ParD

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