DEVELOPMENT OF AMPHIPATHIC CELL-PENETRATING FOLDAMERS FOR DELIVERY OF BIOMACROMOLECULES

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Cell-penetrating peptides (CPPs) are receiving much attention as an intracellular delivery tool for hydrophilic cargo molecules such as drugs, proteins, and nucleic acids. It has been reported that CPPs contain a high relative abundance of positively charged amino acids such as arginine (Arg), and the interaction between cationic side chains of Arg residues and acidic groups existing on the cell surface is essential for cell membrane permeability. On the other hand, the relationship between secondary structures of CPPs and their cell membrane permeability still remains unclear. Previously we reported that amphipathic Arg rich helical peptides whose helical structures were stabilized with hydrophobic 2-aminoisobutyric acid (Aib) residues, penetrated cells more effectively than non-helical Arg-rich peptides. However, these helical peptides showed lower cell permeability than nonaarginine (R9) because replacement of cationic Arg with hydrophobic Aib reduced in the number of Arg and/or changed distribution of Arg residues in peptide. We also reported that $(Leu\text{-}Leu\text{-}Aib)_n$-peptides (**1**) are capable of acting as a useful helical promoter. Based on this knowledge, we hypothesized that conjugation of helical peptide **1** with R9 could stabilize the helical structure and enhance cell membrane permeability. Herein, we designed and synthesized an R9-based foldamers [**Block3**: FAM-bAla-$(Leu\text{-}Leu\text{-}Aib)_3$-$(Gly)_3$-$(Arg)_9$, FAM: 5(6)-carboxyfluorecein]. At first, we performed preferred secondary structural analysis of **Block3** using CD spectra. As a result, **Block3** showed negative maxima around 208 and 222 nm indicating **Block3** formed a stable helical structure whereas R9 did random structures. Furthermore, we evaluated effects of the peptides on delivery of the plasmid DNA (pDNA) or siRNA into cells. MCF-7 cells were treated with the complex of peptide and pDNA or siRNA, and the target gene expression were evaluated by reporter gene luciferase assay and western blotting analysis, respectively. As a result, **Block3** efficiently deliver the pDNA and siRNA into cells. These results suggest that **Block3** could be a candidate for a novel intracellular delivery tool.