Cell-penetrating peptide nanocomplexes enhanced cellular uptake of dsRNA in Sf9 cell line

Narita Thungsatianpun[1], Rapeepat Mavichak[2], Nattanan T-Thienprasert[1], Sasimanas Unajak[1] and Chomdao Sinthuvanich*[1,3]

[1]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand
[2]Aquatic Animal Health Research Center, Charoen Pokphand Foods Public Company Limited, Samutsakorn, Thailand
[3]Specialized center of Rubber and Polymer Materials in agriculture and industry (RPM), Faculty of Science, Kasetsart University, Bangkok, Thailand
*Corresponding author; E-mail: chomdao.si@ku.th

Abstract

The use of double-stranded RNA (dsRNA) to knock down genes of interest has gained increased attention in arthropods for applications in insect pest management and vaccine development for aquatic animals. However, its large size and highly anionic character impede the internalization of dsRNA into cells. To improve cellular uptake, we utilized cell-penetrating peptides (CPPs) as a delivery vehicle to carry dsRNA across the cell membrane. Here, nanocomplexes prepared from 600-bp dsRNA and CPPs, TAT or EB1, were characterized and their ability to carry dsRNA into cells was investigated. The optimal positive to negative charge (P/N) ratio between CPPs and dsRNA was determined by electrophoresis mobility shift assay and fluorescence spectroscopy. Hydrodynamic size and zeta potential of the complexes were assessed by the dynamic light scattering technique. Morphology and size distribution of nanocomplexes were examined by transmission electron microscope. Cellular uptake of CPP/dsRNA nanocomplexes was evaluated in *Spodopteria frugiperda* (Sf9) cell line. Internalized dsRNA levels were assessed by semi-quantitative reverse transcription (RT)-PCR after 1, 6, and 48 h. The results showed that at an appropriate charge ratio, cationic complexes can be formed with size in the range of nanometers. Interestingly, regardless of CPPs used, the 600-bp dsRNA were internalized into the cell during the first hour of incubation. However, levels of dsRNA delivered by TAT were diminished, comparing to EB1 after 48 h. Overall, this work provides more insights into the factors involving nucleic acid delivery in arthropod cells.

Keywords**:** *cell-penetrating peptides, double-stranded RNA, cellular uptake, nanocomplex, Spodopteria frugiperda*