Cell-Penetrating Peptides: How They Facilitate the Transmembrane Transport of Genes and Drugs

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Cell-penetrating peptides (CPPs) are molecules of commonly polycationic nature that traverse the membrane of biological cells within seconds to minutes. During their cellular uptake, they can take other molecules such as drugs, plasmid DNA, siRNA or enzymes along with that would not cross the cell membrane otherwise. Despite their great potential as a helper molecule for drug delivery and gene transfer, their mode of membrane translocation is still mysterious (*1*). It has even been argued that the observed transport across the cell membrane is an artifact caused by chemical fixation of cells (*2*), a common preparation procedure used in microscopy.

Here, we omit the need for chemical fixation by observing the uptake of a fluorescently labeled CPP into living cells with time-lapse confocal microscopy. We observe that a fluorescently labeled CPP, derived from the HIV-1 protein Tat, enters the cytoplasm and nucleus of fibroblasts within seconds, arguing against the suggested artifact of cell fixation. Using differential interference contrast microscopy, dense aggregates are detected on the cell surface. Several observations suggest that these aggregates consist of the CPP bound to membrane-associated glycosaminoglycans (GAGs) such as heparan sulfate (HS). These aggregates grow in parallel with the CPP uptake and are detected only on fibroblasts showing CPP uptake. These observations resemble earlier reports of “capping” of cell surface molecules combined with a polarized endocytotic flow and stress fiber formation. Enzymatic removal of extracellular HS reduced the rate of both CPP uptake and aggregate formation, demonstrating that HS is involved in the uptake mechanism. The binding and clustering of GAGs with CPPs was also evidenced in vitro with light scattering techniques.

We therefore measured the thermodynamic binding parameters for the interaction of the CPP with GAGs and lipids by using isothermal titration calorimetry. The CPP binds much stronger to GAGs (Kd = 0.1-2 microM for various CPPs and GAGs tested) than to negatively charged membrane lipids (Kd = 83 microM). Comparing the binding affinity of the CPP for various cargos (DNA or drugs), extracellular GAGs, and intracellular compounds contributes to better understand the delicate balance between stability of the transport complex, optimum molar ratio for cellular uptake and intracellular release of the cargo.

References:

1. Richard, J. P., *et al.* (2003) *J Biol Chem 278*, 585-90.
2. Lundberg, M., and Johansson, M. (2001) *Nat Biotechnol 19*, 713-4.