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Attenuated Cationic Lytic Peptides for Intracellular Delivery

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Introduction

Intracellular delivery of biomacromolecules is one of the most important topics in current peptide research. Although many approaches have been developed, more efficient methods for the delivery of high molecular weight proteins, including immunoglobulin G (IgG), are still awaited. Cationic amphiphilic peptides often have potent membranolytic activity. If the lytic activity of these peptides can be properly controlled, they could be used as a novel means of intracellular delivery. Since the hydrophobic interaction of peptides with the membrane is very important for membrane lysis, the placement of negatively charged Glu residues in the potential hydrophobic region of the peptides could be used as a switching device to modulate membrane lysis. This simple idea was the impetus for our efforts to develop a new class of delivery peptides, i.e., attenuated cationic lytic (ACAL) peptides.

Results and Discussion

First, we investigated the lytic activity of various amphiphilic peptides, and California wolf spider toxin-derived M-lycotoxin was chosen as the design platform. The mutant peptide L17E, in which the leucine at position 17 was replaced by glutamic acid (IWLTALKFLGKHAAKHEAKQQLSKL amide), was designed [1]. The EC_{50} value (concentration at which 50% of cells die) of L17E was reduced to >40 μM compared to that of M-lycotoxin (1.36 μM). Treatment of cells with model macromolecules, polydextran (10 kDa) and IgG (~150 kDa), in the presence of L17E resulted in marked cytosolic translocation of these molecules in 50% of cells. Efficient intracellular delivery of a ribosome-inactivation protein (saporin), Cre recombinase and IgG delivery was thus achieved, resulting in a specific recognition of cytosolic proteins and subsequent suppression of the glucocorticoid receptor-mediated transcription. The increased hydrophobicity of L17E improved the low pH helical structure and cytosolic delivery efficiency (HAad: IWLTALKFLGKAAAKA-XAKQXLSKL amide; X = L-2-aminoadipic acid (Aad)), resulting in cytosolic translocation of IgG in 75% of cells, an increase of approximately 25% over L17E [2] (Figure 1).

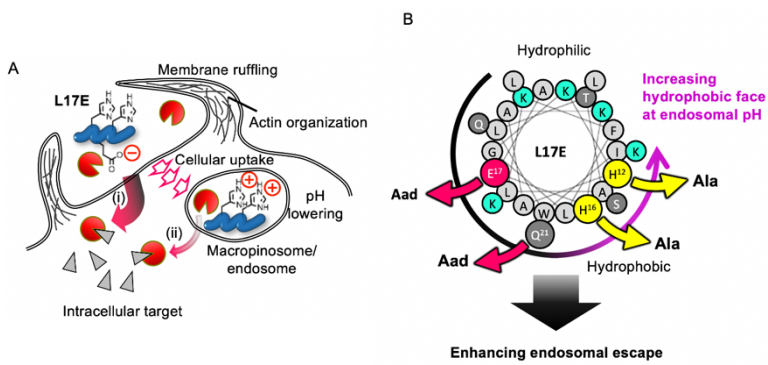

Fig. 1. (A) Schematic representation of the mode of intracellular delivery by L17E. L17E was found to have an ability to induce membrane ruffling, leading to transient permeabilization of membranes at early stages of endocytosis and cytosolic translocation of biomacromolecules (route (i)). However, endosomal escape (route (ii)) did not play a significant role in attaining cytosolic translocation as originally intended [3]. (B) Helical wheel projection of L17E showing substitutions of His^{12} and His^{16} to Ala, and Glu^{17} and Gln^{21} to Aad, yielding HAad. Substitutions led to the enlargement of the potential hydrophobic face in endosomes and enhanced delivery using both routes (i) and (ii). Reprinted with permission from ref. [2].

Conjugation of HAad with pyrene butyric acid as a membrane anchoring moiety (pBu-HAad) further improved the release efficiency. pBu-HAad achieved a comparable level of protein release efficiency into cells with only 1/20 of the HAad concentration [4]. In contrast, conjugates with cholesteryl hemisuccinate and aliphatic fatty acids did not yield a marked improvement. The results of the time-laps microscopic observation and inhibitor studies indicate that membrane anchoring of HAad by a pyrene moiety results in enhanced peptide-membrane interaction and loosening of the lipid packing, which facilitates cytosolic translocation of proteins.

More sophisticated IgG delivery systems based on L17E and other ACAL peptides can be created by appropriate means of complexation or packaging, since IgG must be localized with membrane-permeabilizing ACAL peptides to achieve cytosolic translocation. We therefore sought to formulate IgG with the ACAL peptide L17E to facilitate more efficient permeation of IgG across membranes, using a trimer of L17E. To facilitate complex formation with IgG, the trimer was tagged with an Fc binding peptide. To assess cytosolic translocation, IgG was fluorescently labeled with Alexa Fluor 488 (IgG-Alexa488). Notably, mixing $FcB(L17E)_3$ with IgG-Alexa488 resulted in the formation of liquid droplets or coacervate, which allowed efficient cytosolic translocation of IgG [5] (Figure 2). The addition of negative charges on IgG by modification with Alexa Fluor 488 was crucial for liquid droplet formation. This liquid droplet-mediated intracellular translocation of IgG was not achieved by simple pore formation in the cell membrane. The need for energy-dependent, actin-driven membrane dynamics triggered by the liquid droplet was suggested by pharmacological inhibition experiments. The potential applicability of this approach to other proteins modified with negatively charged molecules was illustrated by the successful delivery of an antibody to the nuclear pore complex (modified with Alexa Fluor 594) and an antibody to mCherry nanobody (labeled with a supernegatively charged green fluorescent protein ((-30)GFP).

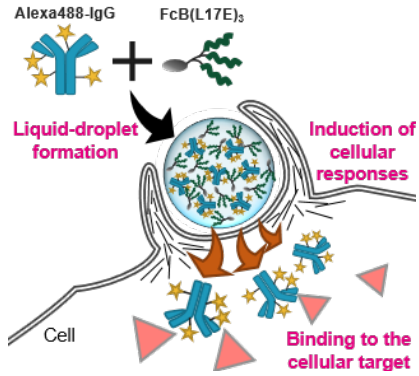

Fig. 2. Possible mechanism of liquid droplet formation and facile cytosolic translocation of IgG in the presence of the trimer of L17E. Reprinted with permission from ref. [5].

Acknowledgments

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