"Co-endocytic" delivery of proteins via artificial receptor/ligand interaction on cell surface

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Statement of Purpose: Intracellular delivery of biomolecules such as DNA, RNA, and protein is important as a basic procedure for biochemistry and biology. Among them protein is difficult to deliver because of various nature and instability of proteins. Many protein delivery reagents have been reported so far and they possess cationic charge both for membrane penetration and complexation with protein. However, these cationic reagents are difficult to apply to less anionic and positive proteins. To apply to wide variety of proteins, modification of artificial receptor on the cell surface will be promising approach. Cellular receptors are taken up by endocytosis responding to the binding of ligand to induce intracellular signaling. The proteins existing on the cell surface such as lipid and GPI anchored proteins can be incorporated together. Thus, artificial receptors which do not induce intracellular signaling for endocytosis will be taken up together with endocytosis (Figure 1). Here we report polymeric receptor molecules carrying many alkyl chains for stable anchoring on the cell membrane. These molecules successfully delivered proteins into cells via co-endocytosis.

Methods: Receptor polymers were synthesized based on our previous method. Briefly, dextran (40k) was modified with ethylenediamine using carbonyldiimidazole, then palmitoyl (hydrophobic anchor), biotin (receptor), and FITC were modified to amine group of ethylenediamine. The content of each group per glucose unit was determined by ^{1}H NMR.

Results: The obtained polymer was modified onto K562 cell by mixing the polymer solution with cell dispersion in PBS for 30 min at 4^{o}C. The fluorescence resulting from FITC labeled onto the polymer was observed from cell surface. This means successful modification of the polymer on the cell surface. The amount of polymer chains modified on one cell saturated when the polymer concentration exceeded 0.1 mg/mL.

The polymer modified on the surface is incorporated into cell during incubation at 37 ^{o}C in serum containing medium. The fluorescence intensity from inside of the cell increased and that from cell membrane decreased with time. The compartment observed inside of the cell can be co-stained by LysoTracker Red, indicating that the polymer was incorporated by endocytic pathway and mainly existed in the endosome or lysosome of the cell. It is important to note that the polymer is not toxic even at high concentration of modification. Thus, the co-endocytosis is less toxic method comparing with cationic lipid-mediated endocytosis.

To introduce streptavidin (SA) into cell, SA was mixed with cells modified with the receptor polymer at 4 ^{o}C, then incubated at 37 ^{o}C. A fluorescence microscopic observation showed a colocalization of SA and polymer on the cell surface, indicating successful recognition of biotin modified on polymer by SA on the cell surface. Fluorescence started to appear from inside of the cell from 1hour of incubation at 37^{o}C. With increasing time, fluorescence from inside became enhanced while the fluorescence from cell membrane became weaker. This result indicates the cellular uptake of SA/polymer complex by endocytosis. Figure 2 shows fluorescence cytometry results after SA introduction. The introduction efficiency is higher than commercially available reagents. It is important to note that the presence of serum shows almost no effect on the amount of the cellular uptake of complex. The uptake of complex was negligible in the absence of polymer.

Conclusions: Artificial receptor polymers modified with palmitoyl groups stably exist on the cell surface by multivalent interaction with cell membrane. The SA/polymer complex formed on the cell surface successfully introduced into cell. The introduction efficiency of SA by the co-endocytic pathway is much higher than commercial protein delivery reagents.

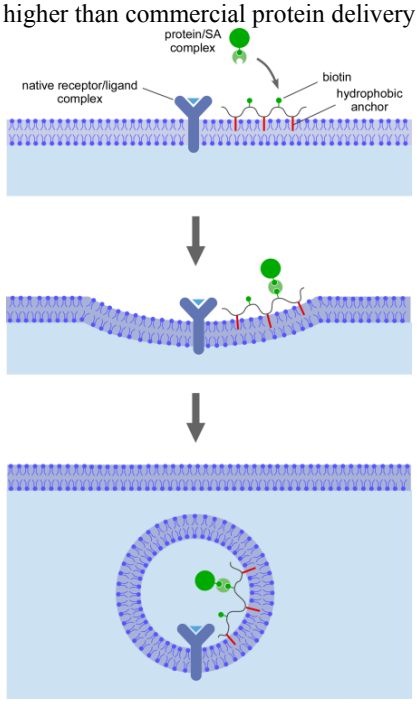

Figure 1. A mechanism of co-endocytosis.

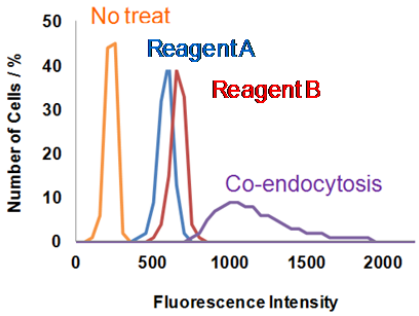

Figure 2. Comparison of protein delivery efficacy.