Artificial Receptor-Mediated Endocytosis for Efficient Protein Delivery into Cells

Takeshi Mori, Kyohei Tobinaga, Cuicui Lee, Masafumi Takeo, Masayoshi Matsuda, Takuro Niidome, Yoshiki Katayama

1 Department of Applied Chemistry, Kyushu University
mori.takeshi.880@m.kyushu-u.ac.jp

ABSTRACT SUMMARY

We developed a intracellular delivery system of proteins via artificial receptor-mediated endocytosis. The artificial receptor which is a polymer with multivalent hydrophobic anchors and biotins was stably modified on cell surface. The receptor successfully bound with complementary proteins and delivered the proteins into cell via endocytosis. The artificial receptor-mediated endocytosis is much effective than commercial reagents for protein delivery.

EXPERIMENTAL METHODS

Receptor polymers were synthesized based on our previous method. Briefly, dextran (40kDa) 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 $^1H$ NMR.

Figure 1. A mechanism of co-endocytosis.

For modification of the polymers on K562 cells ($10^6$ cells/mL), 50 µL of polymer (0.1 mg/mL) dissolved in DPBS was added to the cell suspension and incubated for 30 min with gentle agitation at 4 °C. After cell surface modification, DPBS (2 mL) was added to the mixture and the cells were collected by centrifugation. Then, cells were incubated in medium at 37 °C under 5 % CO₂.

INTRODUCTION

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 a 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 via endocytosis. 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.
RESULTS AND DISCUSSION

The obtained polymer was modified onto K562 cell by mixing the polymer solution with cell dispersion in PBS for 30 min at 4°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 °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 °C, then incubated at 37 °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 1 hour of incubation at 37°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 microscopic observation of uptake of SA by cells which are modified or not modified by polymers after 24 hours.

Figure 3 shows fluorescence cytometry results after SA introduction. The introduction efficiency is higher than commercially available reagents A and B. 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.

CONCLUSION

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.

REFERENCES


ACKNOWLEDGMENTS

We thank the financial support of a Grant-in-Aid for Young Scientists (B) (23700539) from the Ministry of Education, Culture, Sports, Science and Technology.